Identification of new SUMO activating enzyme 1 inhibitors using virtual screening and scaffold hopping

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ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Sumoylation

SUMO

SUMO E1 inhibitor

Virtual screening

Scaffold hopping

ABSTRACT

Sumoylation involves the enzymatic conjugation of small ubiquitin-like modifier (SUMO) protein to their substrate proteins. Sumoylation is not only crucial for maintaining normal cellular physiology but also implicated in the development of several diseases including cancer. SUMO E1, the first protein in sumoylation pathway is of particular significance due to its confirmed role in tumorigenesis. However, notwithstanding its role as potential drug target, only a few small molecule inhibitors of SUMO E1 have been identified. Here, we report the identification of pyrazole and thiazole urea containing compounds as inhibitors of SUMO E1. We have utilized 3D-shape matching, electrostatic potential similarity evaluations and molecular docking to scaffold hop from previously known aryl urea scaffold with SUMO E1 activity to thiazole and pyrazole urea based scaffolds. These two classes of compounds were found to have moderate SUMO E1 inhibitory activity and can be used as starting points for the development of highly potent lead compounds against cancer.

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Proteins undergo a variety of enzyme-catalyzed modifications during or after protein synthesis. These post-translation modifications are crucial for normal cellular physiology and more than 400 such modifications have been described.^{1,2} Most common post-translational modifications include methylation, acetylation, phosphorylation, glycosylation, ubiquitination and sumoylation. Sumoylation involves the reversible modification of target proteins with a small ubiquitin-like modifier (SUMO) protein. Sumoylation is an important mechanism regulating the activities of various proteins involved in cellular processes like DNA replication and repair, chromosome packing and dynamics, genome integrity, nuclear transport, signal transduction and cell proliferation.³⁻⁷ Sumoylation is a reversible and highly regulated pathway and requires the sequential action of three enzymes: an activating enzyme (SUMO E1), a conjugating enzyme (SUMO E2 or Ubc9) and a ligase (SUMO E3) to covalently attach SUMO proteins to the ε-amino group of specific lysine residues in target proteins.⁶⁻⁹ SUMO E1 is a heterodimer of two subunits (Uba2/SAE2 and Aos1/SAE1) and catalyzes the first step of sumoylation pathway that requires the adenylation of SUMO C-terminus and formation of SUMO-AMP conjugate at the expense of ATP.^{10,11} Subsequently SUMO E1 catalytic residue Cys173 and SUMO-AMP intermediate come in close proximity as a result of large conformational change in SUMO E1.¹² Finally, the SUMO protein is covalently attached to SUMO E1 by the formation of a thioester bond between SUMO C-terminus and Cys173 (Figure 1).

Sumoylation has been implicated in many diseases, such as cancer, diabetes, heart and neurodegenerative diseases.^{5,9,13-20} Among the sumoylation proteins, higher expression of SUMO E1 has been observed in several cancers.^{21,22} One recent study confirmed the role of SUMO E1 in the maintenance of Myc tumorigenic phenotype and suggested SUMO E1 as viable therapeutic target in Myc overexpressed cancers.²² Another study showed that the knockdown of SUMO E1 subunit 2 (Uba2 or SAE2) suppresses cancer malignancy and enhances chemotherapy sensitivity in small cell lung cancer.²³ Reduced proliferation of human cancer cells was also observed following the short hairpin RNA based knockdown of SAE2.²⁴

Figure 1. SUMO E1 in sumoylation pathway.

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Although several studies suggested that SUMO E1 can be an attractive target but notwithstanding its role in the development of human diseases, inhibitor discovery efforts were limited and only a few inhibitors have been identified.²⁵⁻²⁹ Adenoviral protein Gam1 that blocked both in vitro and in vivo sumoylation was among the first SUMO E1 inhibitors.²⁶ Other protein-based inhibitors include the mimics of adenylate intermediate or the tetrahedral intermediate generated during thioester bond formation.²⁵ Crystal structures of these mechanism-based inhibitors with SUMO E1 were also reported.¹² NEDD8 E1 inhibitor MLN4924, an AMP mimic was also shown to inhibit SUMO E1.²⁷ Furthermore, high throughput screening of botanical extracts resulted in the identification of natural products ginkgolic acid, anacardic acid and kerriamycin B that inhibited SUMO E1 by blocking the formation of SUMO E1-SUMO intermediate.^{28, 29} We have previously reported phenyl urea and quinazolinyl biaryl urea as non-natural product inhibitors of SUMO E1.^{30, 31} Although both class of inhibitors displayed moderate inhibition of SUMO E1, they were the only typical synthetic small molecules SUMO E1 inhibitor reported to date.

Initially, with a goal to identify new chemical scaffolds with SUMO E1 inhibitory activity, we utilized compound **1** (Figure 2) previously reported by us³¹ as a template for scaffold hopping. Shape and electrostatic potential matching calculations were used to retrieve compounds endowed with similar chemical features but a different scaffold from compound **1**. Namiki shoji small molecular collection (www.namiki-s.co.jp) containing about 4 million purchasable compounds was used as the screening library. At the outset, shape comparisons were performed using ROCS.²⁹ EON program³⁰ was then used to perform electrostatic potential matching calculations on the top ranking 100 compounds from shape similarity. As expected the top ranking

Figure 2. (a) Structures of compounds **1** and **2**; (b) concentration dependent inhibition of compound **2**; (c) alignment of compound **2** (green atom color) with compound **1** (cyan atom color); (d) molecular surface and (e) electrostatic potential of compounds **1** and **2**.

five compounds belonged to quinazolinyl biaryl urea. Other than this scaffold, compound **2** was the top-ranking compound (6th in ranking) with EON ET_Comboscore (a combined score for electrostatics and shape overlap) of 1.54 and ROCS TanimotoCombo (which is a combined Tanimoto coefficient³² for overlap of shape and chemical features) of 1.21 (both in the scale of 0 to 2). As compound **2** shares O-methyl and O-cyclopentyl substituted phenyl urea substructure with compound **1**, significant overlap in shape and electrostatic potential field was observed (Figure 2). Major differences were observed in the other half of the molecule. A thiazole moiety replaced the pyridine ring in compound **2** while the chloro substituted quinazolinyl ring was substituted by a methyl and chloro

substituted phenyl ring. The experimental validation of compound **2** using in vitro sumoylation assay²⁸ (Figure 2) revealed moderate SUMO E1 inhibitory activity. Compound **2** inhibited the sumoylation of substrate RanGAP1 in a concentration dependent manner with a half maximal inhibitory concentration (IC₅₀) value of 92.6 μM (Figure 2).

To analyze the binding of compound **2** with SUMO E1, molecular docking using Glide program³³⁻³⁵ was employed in the extra precision mode (Glide-XP). We hypothesized that this compound competes with ATP binding, so SUMO E1 ATP binding pocket located close to the interface of two subunits (Aos1 and Uba2) of SUMO E1 was utilized for docking. Moreover, thiazole urea is a common kinase inhibitor scaffold and competes with ATP for its inhibitory activity.^{36, 37} SUMO E1 co-crystal structure with bound ATP (PDB code 1Y8Q) was used for molecular docking. As shown in Figure 3, compound **2** occupies the ATP binding pocket with its thiazole ring overlapping with the sugar ring. The chloro and methyl substituted phenyl ring in compound **2** is predicted to occupy the position of adenine ring in ATP as in the case of SUMO E1 crystal structure (PDB code 1Y8Q). Compound **2** is predicted to interact with SUMO E2 via hydrogen bonding and hydrophobic interactions and form similar interactions as ATP. Specifically predicted binding mode outlines hydrophobic contacts with Leu49, Ile96, Leu116 residues and hydrogen-bonding interaction with Arg59 as displayed by ATP in SUMO E1 ATP crystal structure (PDB code 1Y8Q).

Figure 3 (a) Molecular docking predicted binding pose of compound **2** (green atom color) in SUMO E1 ATP binding pocket. ATP is shown in grey atom color. (b) Interaction details of compound **2**.

In order to identify thiazole urea scaffold containing compounds with better SUMO E1 inhibitory activity, structurally related compounds were identified by carrying out 2D structural similarity search against Namiki shoji collection of commercially available compounds. Compound **2** was used as a query to retrieve compounds utilizing MACCS structural keys. A Tanimoto coefficient³² cut-off of 0.7 was used. About 340 structurally similar compounds with thiazole urea scaffold were obtained. Further prioritization of these hits was accomplished using molecular docking calculations employing Glide-XP program.^{33-35, 38} The SUMO E1 ATP binding site was selected as the target area for molecular docking. The docking scores for these 340 compounds ranged from -7.4 to -1.86. All compounds were then analyzed visually for the interactions they make with SUMO E1 active site residues as the intuition and experience of researchers play a very important role in selecting good compounds³⁹. The docking predicted binding mode of compound **2** and co-crystal structure of ATP bound SUMO E1 (PDB code 1Y8Q) were used as reference structures for selecting compounds making good contacts with the protein. The compounds displaying similar binding mode as compound **2** were prioritized. Specifically, compounds making hydrophobic contacts with

Leu49, Ile96 and Leu116 and hydrogen-bonding interaction with Arg59 were given high priority. Additionally, in-stock availability from commercial vendors and costs were also considered while selecting compounds for biological assay. Finally, a set of 22 compounds were acquired from commercial vendors for evaluation of SUMO E1 inhibitory activity using an in vitro sumoylation assay.²⁸ The vendors have verified that each compound had more than 90% purity. The chemical structure and purchase information from commercial vendors have been given as supplementary information Table S1. Initially, these 22 compounds were evaluated for their ability to inhibit the sumoylation of substrate RanGAP1 at concentrations of 100 and 20 μ M. Ten μ M ginkgolic acid (GA) was used as a positive control for the experiment. As shown in Figure 4, nine out of 22 compounds inhibited at least 60 % of the SUMO E1 activity at the concentration of 100 μ M. There was one compound (compound **8**) that displayed more than 60 % inhibitory activity at 20 μ M. Structure activity analyses of tested compounds revealed that substitution of benzothiazole in place of thiazole ring was detrimental to the activity. Compounds **4**, **9**, **10**, **11** and **23** with benzothiazole ring in place of thiazole were either inactive or less active. This reduction in activity may be due to increased rigidity in the compounds and thereby decreasing its polar surface area. The 4-position of thiazole ring preferred aromatic substitutions and replacement with acetyl group resulted in reduced activity as in the case of compounds **17** and **21**. At this position, phenyl ring with meta or para substituted chloro and methyl groups were the most productive substitution (compounds **2**, **3**, **14** and **15**) while substitution of phenyl ring with methoxy had negative effect on the activity (compounds **19** and **20**). Phenyl ring on the other side of urea moiety preferred alkyloxy and aryloxy substitutions whereas substitution of halogens reduced the biological activity (compounds **13**, **16**, **18** and **24**). Compound **8** was more active than the parent compound **2** and was subsequently evaluated in a concentration dependent experiment. Compound **8** inhibited the sumoylation of substrate RanGAP1 in a concentration dependent manner with IC₅₀ of 33.2 μ M (Table 1).

Figure 4. SUMO E1 inhibitory activities of (a) thiazole urea derivatives and (b) pyrazole urea derivatives. 10 μ M ginkgolic acid (GA) was used as a positive control.

Despite showing distinct chemical features than previously reported phenyl and quinoxaliny urea compounds^{30, 31}, compound **8** was only moderately active and not better than compound **1**. Furthermore, several structurally related compounds with thiazole urea scaffold were less potent (Figure

4) and activity could not be improved. Therefore, we investigated whether the pyridine ring in compound **1** or thiazole core in reported compounds could be replaced by a bioisostere that would display similar properties. Pyrazole ring have been successfully used in scaffold hopping studies to replace pyridine and thiazole ring.^{36, 40} In order to obtain compounds with pyrazole ring in place of pyridine or thiazole ring, a substructure search for pyrazole urea scaffold was carried out that revealed 133 compounds in Namiki shoji collection. These compounds were further prioritized using shape and electrostatic matching calculations. Compound **1** was again used as a query to filter pyrazole urea scaffold compounds with similar shape and electrostatic properties. Shape similarity between compound **1** and each molecule in Namiki shoji collection of commercially available compounds was calculated using ROCS program. TanimotoCombo was used to rank-order compounds. All 133 compounds were also evaluated for their similarity in electrostatic potential field with compound **1** using EON program. ET_combo score was used to score compounds and the top ranking 100 compounds were chosen for further analysis. Further prioritization of these 100 hits with shape and electrostatic potential similarities with compound **1** was accomplished using molecular docking calculations employing Glide-XP program.^{33-35, 38} The SUMO E1 ATP binding site was selected as the target area for molecular docking and all compounds were ranked using Glide-XP scoring function. The docking scores for these 100 compounds ranged from -8.074 to -4.652. Binding poses for these 100 hits were analyzed visually for the interactions they make with SUMO E1 ATP binding site similarly as in the case of thiazole urea based compounds. Finally based on 3D shape and electrostatic potential similarity with compound **1**, molecular docking, visual inspection and in-stock availability, a set of 18 pyrazole urea compounds was purchased from commercial vendors for biological assay (supplementary information Table S2). These 18 compounds were tested for their ability to inhibit the sumoylation of substrate

Figure 5. (a) alignment of compound 39 (yellow atom color) and compound 41 (magenta atom color) with compound 1 (cyan atom color); (b) molecular surface; (c) electrostatic potential of compounds 39 and 41; (d) molecular docking predicted binding mode compounds 39 and 41. ATP is shown in grey atom color. (e) interaction details of compound 39 and (f) interaction details of compound 41.

RanGAP1 in the similar manner as the previously tested thiazole urea compounds. As shown in Figure 4, 9 out of 18 compounds inhibited at least 60 % of the SUMO E1 activity at the concentration of 100 μ M. Four compounds (compounds **37**, **39**, **41**) displayed more than 40 % inhibitory activity at 20 μ M.

Table 1. Structure and inhibitory activities of the most potent virtual screening hits

Cmpd #	Structure	IC ₅₀	M _w ^a	HBA ^b	HBD ^c	Nrot ^d	QP logPo/w ^e	QP logHERG ^f	QP Caco2 ^g	QP logBB ^h	QP logKhsa ⁱ
1		13.4	505.9	7	2	9	5.03	-6.119	823.73	-0.93	0.64
2		92.6	472.0	4	2	8	5.65	-5.15	1574.67	-0.31	0.97
8		33.2	442.5	6	2	8	3.69	-5.18	1260.47	-0.42	0.12
37		>100	444.4	5	2	7	3.32	-5.11	443.89	-0.96	0.27
39		13.8	456.5	3	2	8	4.98	-5.57	442.62	-1.14	0.93
40		73.0	446.5	5	2	8	3.55	-5.41	388.18	-1.18	0.34
41		30.4	430.4	5	2	6	2.96	-5.17	389.16	-0.97	0.18

^a Molecular weight. ^b Hydrogen bond acceptor. ^c Hydrogen bond acceptor. ^d Number of rotatable bonds. ^e Log of the octanol/water partition coefficient, range 95% of drugs (−2.0 – 6.5). ^f Predicted IC₅₀ value for blockage of HERG K⁺ channels, range 95% of drugs (concern below −5). ^g Caco2 cell permeability in nm/s, range 95% of drugs (<25 poor, >500 great). ^h Predicted brain/blood partition coefficient, range 95% of drugs (−3.0 – 1.2). ⁱ Prediction of binding to human serum albumin, range 95% of drugs (−1.5 – 1.5).

Structure activity analyses of tested compounds revealed that non-aromatic substituents were not preferred at 3-position of pyrazol ring and substitution of tertiary butyl and methyl in compounds **25-27** and **32-36** led to decreased activity. Whereas substitution of aromatic ring improved the activity as in compounds **29-31** and **37-42**. Compounds with oxo-pyrimidinyl ring substitution at 1-position of pyrazol ring (compounds **37-42**) were more active than compounds with methyl, cyclopentyl, aryl and aryl halide substitutions (compounds **25-35**). Substitution at

5-position of oxo-pyrimidinyl ring has negative effect on activity (compound **36**) while no functional group at this position improved the biological activity. Compounds **37**, **39-41** that displayed more than 40 % inhibitory activity at 20 μ M as illustrated in Table 1 were then subsequently evaluated in concentration dependent experiments and IC₅₀ values and their structures are presented in Table 1. Compounds **39** and **41** were found to be the most potent with IC₅₀ values of 13.8 and 30.4 μ M respectively. The activity of compound **37** was found to be

inconsistent. Despite showing reasonable activity at concentrations of 100 and 20 μ M (Figure 4 and supplementary information Table S2), it was found inactive when tested in dose dependent experiment. The large variation in activities of compound **37** on sumoylation may be related to its stability. The inhibitory activities of compounds **39** and **41** are comparable to the quinazolinyl-oxo biaryl urea compound previously reported by us (compound **1**). However, these compounds belong to a novel chemical class displaying SUMO E1 inhibitory activities. Both compounds exhibit similar shape as compound **1** (Figure 5a and 5b) with ROCS TanimotoCombo of 0.789 and 0.927 for compounds **39** and **41** respectively. Both compounds also exhibited similar electrostatic potential with ET_Combo score of 1.072 and 1.026 for compounds **39** and **41** respectively (Figure 5c). Molecular docking revealed that both compounds occupy the ATP binding pocket of SUMO E1 as predicted for compound **1**³¹ and **2** (Figure 3) with the pyrazole moiety in compounds **39** and **41** nicely overlapping with the ATP sugar ring (Figure 5d). The oxo-pyrimidine moieties in compounds **39** and **41** overlap with the adenine ring of ATP and make hydrophobic contacts with Leu49, Ile96 and Leu116 (Figure 5e and 5f). However, the aryl-urea group in both compounds **39** and **41** is predicted to occupy different regions than that of compound **2**, extending towards the opening of ATP binding pocket near Asn118, Ile343, Trp344 and Asp345. Here the aryl-urea group makes hydrophobic contacts with Ile343 and Trp344 and hydrogen-bonding interactions with Asp50 and Asp117 (Figure 5e and 5f).

Having identified two new scaffolds with moderate SUMO E1 inhibitory activity, we next assessed the pharmacokinetic properties of these scaffolds and compared them with previously reported compounds. QikProp program was used to calculate several properties that are presented in Table 1. All reported compounds fulfill Lipinski's drug-like^{38, 41} properties and no more than one Lipinski violations have been observed. The physicochemical properties of both thiazole and pyrazole based compounds are not in borderline range as seen for compound **1** and other previously reported natural products^{28, 29} and present reasonable scope for optimization of physicochemical properties and potency. None of them contains pan assay interfering substructure⁴² and no reactive or promiscuous compounds were detected according to Elli Lilly rules.⁴³ The aqueous solubility of all reported compounds have been found to be in acceptable range with predicted octanol/water partition coefficient (QPlogPo/w) in 2.96 to 5.65 range (-2 to 6.5 for 95% of oral drugs). All reported compounds were predicted to have IC₅₀ value for the blockage of HERG K⁺ channels (QPlogHERG) > -5 which is the reference value for 95% of oral drugs. The predicted Caco2 cell permeability presented as QPPCaco in Table 1 was found to be in 388.18 to 1574.67 that are in the acceptable range for 95% of oral drugs. The predicted brain/blood partition coefficient (QPlogBB) is also within acceptable range (-3.0 to 1.2 for 95% of oral drugs). None of the compounds is predicted to bind human serum albumin as QPkhsa values, a measure of predicted binding to human serum albumin is within -1.5 and 1.5.

In summary, we have identified thiazole and pyrazole urea based compounds as moderately potent inhibitors of SUMO E1 protein. These compounds were identified using scaffold hopping and virtual screening approaches. The biological activities of reported compounds were comparable to previously reported compounds. Both classes of inhibitors present broad scope for chemical optimization for better drug-like and pharmacokinetic properties and can be used as starting points for development into more potent SUMO E1 inhibitors with therapeutic potential.

Acknowledgments

We thank RIKEN Integrated Cluster of Clusters (RICC) at RIKEN for the supercomputing resources used for this study. We thank members of our labs for help and discussions. This work was supported in part by the Initiative Research Unit program and the Chemical Genomics Research Project, RIKEN, the CREST Research Project, the Japan Science and Technology Corporation, and a Grant-in-Aid for Scientific Research (C) and on Priority Area "Cancer" from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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