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2-(4-Chlorophenyl)-2-oxoethyl 4-benzamidobenzoate derivatives, a novel class of SENP1 inhibitors: Virtual screening, synthesis and biological evaluation

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ABSTRACT

Prostate cancer is one of the most prevalent types of malignant cancers in men and has a high mortality rate among all male cancers. Previous studies have demonstrated that Sentrin/SUMO-specific protease 1 (SENP1) plays an important role in the occurrence and development of prostate cancer, and has been identified as a novel drug target for development of small molecule drugs against prostate cancer. In this paper, we used virtual screening and docking to identify compound **J5** as a novel lead compound inhibiting SENP1, from SPECS library. We further investigated the SAR (structure–activity relationship) of the benzoate substituent of compound **J5**, and discovered compounds **8d** and **8e** as better small molecule inhibitors of SENP1. Both compounds are the high potent SENP1 small molecule inhibitors discovered up to date, and further lead optimization may lead to a series of novel anti-SENP1 agents. Further SAR studies are in process and will be reported in due course.

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Modification of proteins by conjugation of Small Ubiquitin-like Modifier (SUMO) is a key mechanism for regulating many cellular processes.¹⁻⁴ SUMOylation is a dynamic process and can be reversed by SUMO-specific proteases (SENPs), which are critical in maintaining the balance between the level of SUMOylated and unmodified cellular substrates and hence play an important role in mediating normal cellular physiology.⁵⁻⁷ Recently, SENP1 was found to be over-expressed in over 50% of more than 100 prostatectomy cases with high-grade prostatic intraepithelial neoplasia (PIN) and prostate cancer.^{8,9} The role of SENP1 overexpression in the development of prostate cancer has been confirmed in SENP1 transgenic mice.^{10–12} Thus, SENP1 could be the attractive target of prostate cancer. Although some inhibitors designed for SENP1 were developed by us and other groups,^{13–16} novel SENP1 inhibitors with better efficacy are still urgently needed.^{17,18} Thus, the present study was carried out to seek novel leads as inhibitors of SENP1 by (a) virtual screening and (b) structural optimization. Here, we report the process of screen, synthesis, modification and biological evaluation of a novel type of SENP1 inhibitors.

In this study, virtual screening and docking were performed using Glide version 4.5¹⁹ (Schrodinger Suite 2007) with default docking parameter settings. The structure of SENP1 from SENP1-SUMO2-RanGAP1 crystal complex, previously determined by Hay group,²⁰ was retrieved from the Protein Data Bank (PDB entry: 2IYO). Hydrogen atoms and charges were added during a brief relaxation performed using the 'Protein Preparation' module in Maestro with the 'preparation and refinement' option,²¹ and a restrained partial minimization was terminated when the rootmean-square deviation (RMSD) reached a maximum value of 0.3 Å in order to relieve steric clashes of amino acid residues located within 14 Å from the catalytic thiolate of Cys603 were defined as part of the binding site for docking studies. All crystallographic water molecules were removed from the coordinate set. The compound library for screening is from SPECS company [SPECS: Chemistry Solutions for Drug Discovery, 2008; http://www.specs.net/ (accessed October 1, 2011).]. All 180,000 compounds were desalted, neutralized, and parameterized using the OPLS 2005 force field. Then, tautomers and ionization states expected to occur in the pH range of 5.0-9.0 were generated using the 'ionize' module. In the docking process, standard-precision (SP) and extra-precision (XP) docking were respectively adopted to generate the minimized pose, and the Glide scoring function (G-Score) was used to select the final pose with the lowest energy conformation for each ligand.²² Thirty-eight compounds that ranked in

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Figure 1. Concentration gradient of our compound 8d on the inhibition of SUMO-ARanGAP cleavage by SENP1 and its IC₅₀ curve.



Figure 2. Computational model of compound J5 (left) and 8e (right) bound to SENP1 revealed by docking simulations. For protein, surface of SENP1 are shown in grey. Side chains of crucial residues in the binding site are shown as stick and labeled. Hydrogen bonds between compounds and SENP1 are depicted in dotted line in yellow. Figures were generated by Pymol.

the top 100 of both SP and XP scores were eventually purchased and dissolved in DMSO to biological test.

The purification of recombinant SENP1 was performed as described by Hay and co-workers.²⁰ and IC₅₀ values against SENP1 were determined by using our bioassay (Fig. 1) described previously.^{16,23,24} Among these 38 compounds, five compounds show SENP1 inhibition at 50 μ M (Table S1) and compound **J5** was further tested to have the most potent inhibition of SENP1 (IC₅₀ = 2.385 μ M). The docking poses of compound **J5** to the active site of SENP1 are the same in both SP and XP modes. As showed in Figure 2, the shape of compound **J5** is well stuck into the binding site composed by Trp465, Asp468, Phe496, His529, Val532, Gln597 and Cys603. The amide nitrogen and carbonyl oxygen of

compound **J5** form 2 hydrogen bonds with the side chain nitrogen of Gln597 and epsilon 2 nitrogen of His529, respectively, leading to the stability of the extended binding mode of compound **J5** so as to play an inhibiting role in the function of SENP1. More importantly, the binding mode of compound **J5** implies that the pocket around Phe496 and His529 is still unused by the binding of compound **J5**, which could be a potential direction for structural optimization.

Based on our lead compound **J5**, we designed and synthesized a series of derivatives to further explore the SAR (structure–activity relationship) of the 2-(4-chlorophenyl)-2-oxoethyl 4-benzamidobenzoate analogs.

The derivatives of compound **8** were prepared using the methods outlined in Scheme 1. α -Bromo-4-chloroacetophenone (1) was



Scheme 1. Synthesis of compounds 8a-g. Reagents and conditions: (a) 4-Aminobenzoic acid, K₂CO₃ (yield: 80%); (b) RCOOH, SOCl₂; (c) TEA, THF (yield: 8a: 64%; 8b: 76%; 8c: 62%; 8d: 67%; 8e: 72%; 8f: 52%; 8g: 58%)



Scheme 2. Synthesis of compound 8h. Reagents and conditions: (a) SOCl₂ (b) ethyl 4-aminobenzoate, TEA, THF (yield: 75%); (c) Fe powder, NH₄Cl, EtOH, H₂O, cat concd HCl (yield: 80%); (d) NaOH, EtOH; (e) 2-bromo-1-(4-chlorophenyl)ethanone, K₂CO₃ (two steps: 66%).



Scheme 3. Synthesis of compound 8i. Reagents and conditions: (a) 10% Pd/C, H₂, MeOH/THF (yield: 54%).

reacted with 4-aminobenzoic acid (**2**) in the presence of K_2CO_3 to afford compound **3**, which was then reacted with acid derivatives in the presence of TEA to afford compounds **8a–g**. The compounds were purified by silica gel chromatography.

The synthesis of compound **8h** was started from 3-nitrobenzoic acid (**4**) which was converted to the corresponding acid chloride, and then reacted with ethyl-4-aminobenzoate to afford intermediate **5** in a high yield. Reduction of compound **5** afforded compound **6**, which was further hydrolyzed by treatment with aqueous NaOH solution, and the resulting compound **7** was converted to compound **8h** in the presence of K₂CO₃ (Scheme 2). Scheme 3 described the preparation of benzoic acid derivative **8i**, which was prepared by deprotection with 10% Pd/C under H₂ atmosphere.

In the work presented here, we focus on the SAR studies of the ring A, as shown in the Table 1. A number of analogues (compounds **8a–i**) were prepared and representative synthetic routes used to yield compounds are shown in Schemes 1 and 2. In our lead compound **J5**, the meta position of the left benzene ring is substituted by a methoxy. We first designed compound **8c**, in which the methoxy was removed and replaced a hydrogen atom. As tested by using our inhibition assay, compound **8c** showed a slightly decreased potency in inhibiting SENP1 protein. By replacing the methoxy at the meta position with more polar amino and hydroxyl groups, we obtained compounds **8h** and **8i**. However, both compounds lost their potency in inhibiting SENP1 protein completely ($IC_{50} > 50 \mu M$). This result suggests that polar substitutes are not suitable at the meta site of the ring A.

As predicted by our modeling studies, a meta benzene-ring substitute may introduce a perpendicular π - π interaction with the phenyl ring of Phe496, we designed compound **8e**,²⁵ in which the meta methoxy was replaced by a benzoxy. As expected, compound **8e** (IC₅₀ = 1.080 μ M) was more potent than compound **J5** in inhibiting SENP1 protein. Considering that the methoxy is an electron-donating group, we designed compounds **8a**, **8b** and **8d**,²⁶ in which the *meta*-methoxy is replaced with a nitro, a fluorine and a bromine respectively. After replacing with an electron-withdrawing group, all these three compounds showed an improved potency in inhibiting SENP1 protein.

Our modeling studies predicted that a substitute at the para site of the ring A may clash with the phenyl ring of Phe496, leading to a decrease in SENP1 inhibiting potency. To further testify our hypothesis, we designed compounds **8g** and **8f**, in which para and meta sites of the ring A are replaced with a pair of methoxy

Table 1

Binding data for compounds J5 and 8a-i on SENP1



Compound	R ¹	R ²	SENP1 IC ₅₀ (µM)
J5	-OMe	Н	2.385 ± 0.059
8a	NO ₂	Н	1.856 ± 0.205
8b	F	Н	1.735 ± 0.020
8c	Н	Н	3.542 ± 0.007
8d	Br	Н	1.175 ± 0.033
8e	-OCH ₂ Ph	Н	1.080 ± 0.010
8f	-OMe	-OMe	>50
8g	–OEt	–OEt	>50
8h	NH ₂	Н	>50
8i	OH	Н	>50

 IC_{50} values reflect the average from at least three separate experiments.

and ethoxy groups respectively. As expected, both compounds lost their SENP1 inhibiting potency completely ($IC_{50} > 50 \ \mu$ M).

In conclusion, we explored the preliminary SAR of the ring A. In this aromatic ring, its meta site disfavors hydrogen donor substitutes, but a benzoxyl group also bears a promising SENP1 inhibiting activity due to the increase of a perpendicular π – π interaction between its phenyl ring with the phenyl ring of Phe496. An introduction of substitutes at para site based on J5 can result the loss of SENP1 inhibiting potency, due to the spatial clash with Phe496. Further SAR investigations are in progress, and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09. 037.

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- 23. The DNA sequence of the catalytic domain of SENP1 (a.a419–a.a643) (SENP1C) amplified by PCR from PC3 cDNA library was cloned into PET28a(+) vector. The SUMO2-ARanGAP plasmid was a gift from Dr. Jinke Cheng (SJTU, Shanghai China). Both plasmids were transfected into *E. coli* BL21, and the expression of SENP1C was induced with 0.5 mM isopropyl-p-thiogalactoside (IPTG) at 16 °C for 12 h. SUMO2-ARanGAP was induced with 1 mM (IPTG) at 25 °C for 12 h. Cell pellets were resuspended in lysis buffer (300 mM NaCl, 50 mM PBS pH 8.0, 10 mM imidazole, 10 mM β -mercaptoethanol and 10% glycerol) and sonicated. His-taged proteins SENP1C and SUMO2-ARanGAP were purified using Ni-NTA-agarose and eluted with 50–250 mM gradient of imidazole in 300 mM NaCl, 50 mM PBS pH8.0, 10 mM β -mercaptoacetic ethanol and 10% glycerol.
- 24. 5 nM SENP1C was incubated with compounds for 10 min at 37 °C. Then 6 μg SUMO2-ΔRanGAP was added and incubated for another 45 min at 37 °C. The reaction was terminated by adding loading buffer and boiling on heat block for 5 min. The proteins were separated by SDS-PAGE and visualized by coomassie brilliant blue G25.
- Compound **Be**: white solid, yield 58%. ¹H NMR (400 MHz, DMSO-d₆): δ 5.21 (s, 2H); 5.73 (s, 2H); 7.27–7.51 (m, 7H); 7.59–7.68 (m, 4H); 8.00–8.06 (m, 6H); 10.59 (s, 1H). ¹³C NMR (100 MHz, DMSO-d₆) δ 67.3, 69.9, 114.6, 118.7, 120.2, 120.7, 124.2, 128.4, 128.9, 129.5, 130.1, 130.2, 130.8, 133.0, 136.3, 137.2, 139.3, 144.4, 158.8, 165.3, 166.1, 192.5 ppm. HR-ESI-MS: 522.10704 (C₂₉H₂₂ClNO₅, [M+Na⁺])
- Compound **8d**: white solid, yield 54%. ¹H NMR (400 MHz, DMSO-*d*₆): δ 5.73 (s, 2H); 7.51–7.55 (m, 1H); 7.66–7.68 (d, *J* = 8.4 Hz, 2H); 7.82–7.84 (d, *J* = 8 Hz, 1H); 7.98–8.07 (m, 7H); 8.18 (s, 1H); 10.71 (s, 1H). ¹³C NMR (100 MHz, DMSO-*d*₆) δ 67.4, 120.2, 122.4, 127.5, 129.5, 130.2, 130.8, 130.9, 131.2, 133.0, 135.1, 137.1, 139.3, 144.1, 164.9, 165.2, 192.5 ppm. HR-ESI-MS: 493.97708 (C₂₂H₁₅BrCINO₄, [M+Na⁺])