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Discovery of 1-[4-(*N*-benzylamino)phenyl]-3-phenylurea derivatives as non-peptidic selective SUMO-sentrin specific protease (SENP)1 inhibitors

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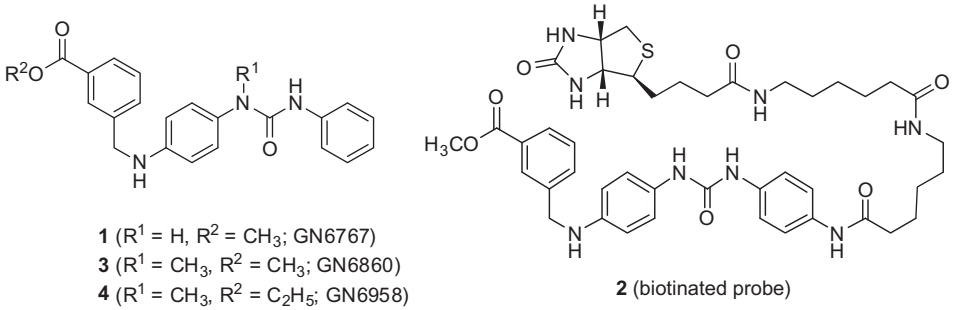
ABSTRACT

We developed 1-[4-(*N*-benzylamino)phenyl]-3-phenylurea derivative **4** (GN6958) as a non-peptidic selective SUMO-sentrin specific protease (SENP)1 protease inhibitor based on the hypoxia inducible factor (HIF)-1α inhibitor **1** (GN6767). The direct interaction of compound **1** with SENP1 protein in cells was observed by the pull-down experiments using the biotin-tagged compound **2** coated on the streptavidin affinity column. Among the various 1-[4-(*N*-benzylamino)phenyl]-3-phenylurea derivatives tested, compounds **3** and **4** suppressed HIF-1α accumulation in a concentration-dependent manner without affecting the expression level of tubulin protein in HeLa cells. Both compounds inhibited SENP1 protease activity in a concentration-dependent manner, and compound **4** exhibited more potent inhibition than compound **3**. Compound **4** exhibited selective inhibition against SENP1 protease activity without inhibiting other protease enzyme activities in vitro.

Small ubiquitin-like modifier (SUMO), a protein that shares about 18% sequence identity with ubiquitin, modulates many biological processes including nuclear transport, transcription, replication, recombination, and chromosome segregation.[1,2] Modification of proteins by SUMO is a dynamic and reversible process and controlled by a series of on/off enzymes. 'SUMOylation', the covalent interaction between the C-terminus of SUMO and the ε-amino group of a lysine residue in the target protein, is mediated by activating (E1),[3,4] conjugating (E2),[3,5] and ligating (E3) enzymes;[6–8] however these are entirely distinct from ubiquitin E1, E2, and E3.[9,10] On the contrary, the 'deSUMOylation' is promoted by a family of SUMO/sentrin specific proteases (SENPs).[11] In the mammalian system, six SENPs (SENPs 1–3 and 5–7) have been reported and, in particular, SENP1, a nuclear protease, deconjugates a large number of SUMOylated proteins.[12] For example, SENP1 has been shown to regulate androgen receptor transactivation by targeting histone deacetylase 1 and induce c-Jun activity through deSUMOylation of p300.[13,14] Moreover, SENP1 is overexpressed in human prostate cancer specimens.[9]



1 (R^1 = H, R^2 = CH_3; GN6767)
3 (R^1 = CH_3, R^2 = CH_3; GN6860)
4 (R^1 = CH_3, R^2 = C_2H_5; GN6958)

2 (biotinated probe)

Figure 1. Structures of 1-[4-(*N*-benzylamino)phenyl]-3-phenylurea derivatives **1**–**4**.

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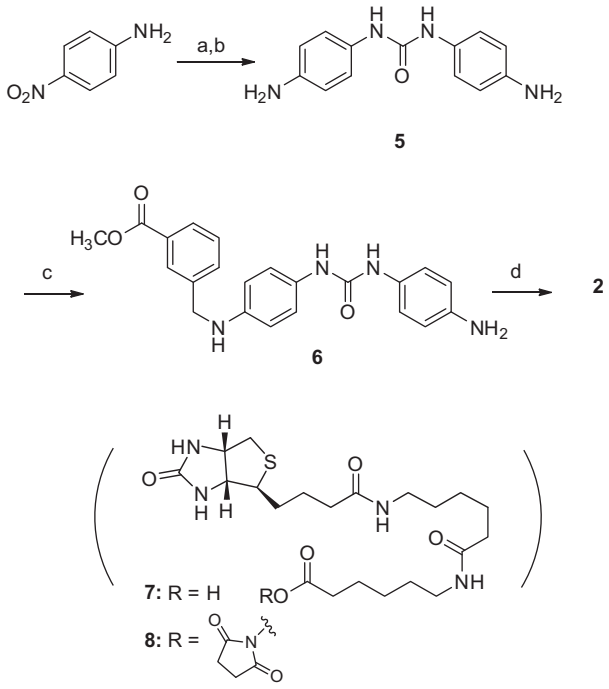
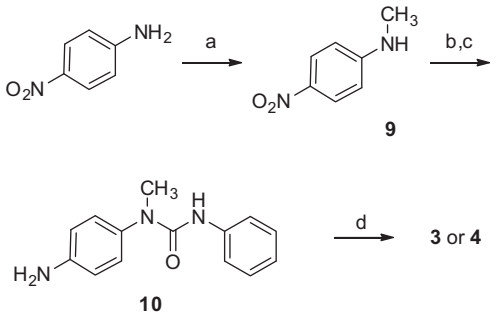


Scheme 1. Synthesis of the biotin-conjugated probe **2**. Reagents: (a) (i) triphosgene, toluene; (ii) 4-nitroaniline, toluene, reflux; (b) H_2, Pd/C, MeOH, 2 steps 63%. (c) methyl benzaldehyde-3-carboxylate, $NaCNBH_3$, MeOH, 10%; (d) **8**, cat. DMAP, $CHCl_3$, 43%.



Scheme 2. Synthesis of the compounds **3** and **4**. Reagents: (a) (i) 1-hydroxymethylbenzotriazole, EtOH; (ii) $NaBH_4$, THF; (b) (i) triphosgene, toluene; (ii) aniline, toluene, reflux; (c) H_2, Pd/C, MeOH, 2 steps 76%; (d) methyl 3-formylbenzoate or ethyl 3-formylbenzoate, $NaCNBH_3$, MeOH.

We have focused our efforts on the development of hypoxia-inducible factor (HIF)-1 inhibitors as pathological angiogenesis inhibitors. HIF-1 is known as a heterodimeric complex consisting of a hypoxically inducible subunit, HIF-1α, and a constitutively expressed subunit, HIF-1β. Under normoxic conditions, HIF-1α protein is subject to oxygen-dependent prolyl hydroxylation, leading to rapid degradation by von Hippel–Lindau tumor suppressor protein (pVHL)-mediated ubiquitin-proteasome system (UPS).[15] Under hypoxic conditions, HIF-1α is not degraded by UPS due to the limited oxygen supply for prolyl hydroxylase (PHD) activity. The stabilized HIF-1α binds to HIF-1β to form a heterodimeric complex, which binds to the hypoxia response element (HRE) DNA sequence with co-activators to activate various genes including angiogenesis factors, such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO).[16] HIF-1α is found at increased levels in a wide variety of human primary cancers compared with corresponding normal tissue. Therefore, HIF-1 has been considered an important target for the development of anticancer agents.[17–21]

We recently reported 1-[4-(*N*-benzylamino)phenyl]-3-phenylurea derivative GN6767 as a new class of HIF-1α inhibitor (com-

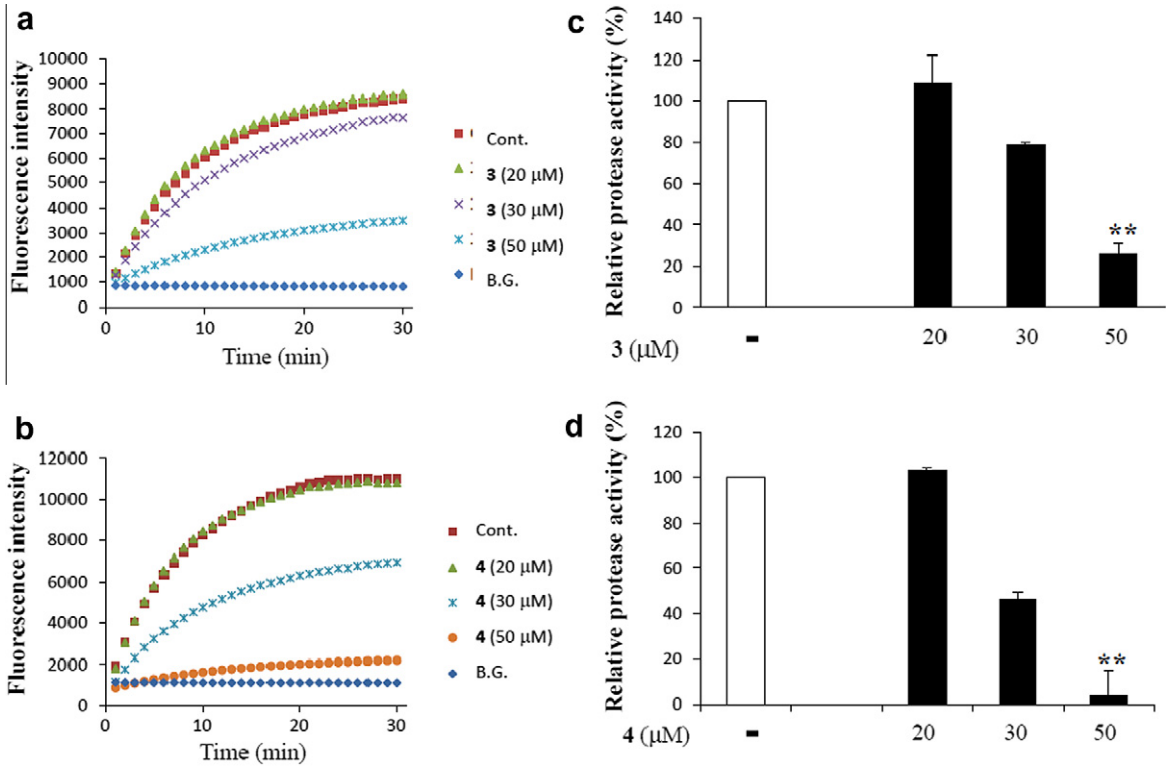


Figure 2. Inhibition of SENP1 catalytic domain (SENP1-CD) endopeptidase activity by compounds **3** and **4**. Compounds were titrated in HEPES buffer (50 mM HEPES, 0.1 mM EDTA, pH 7.9), combined with SENP1-CD (3 nM), and incubated for 10 min in 96-well plates before assaying with the SUMO-1-AMC (300 nM). Fluorescence intensity was plotted on a fluorescence plate reader (excitation/emission wavelengths 380/460 nm; Infinite F200; Tecan) for 30 min after adding the SUMO-1-AMC (a and b). Enzymatic activity was determined as the relative protease activity 5 min after adding the SUMO-1-AMC (c and d). Statistical significance: $^{**}P$ <0.01, compared with control (−).