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In silico identification of poly(ADP-ribose)polymerase-1 inhibitors and their chemosensitizing effects against cisplatin-resistant human gastric cancer cells

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ABSTRACT

Poly(ADP-ribose)polymerase-1 (PARP-1) enzyme is involved in the repair of DNA damages made by certain anticancer agents. It is suggested that PARP-1 inhibitors potentiate the cytotoxic effects and circumvent the resistance of DNA-modifying anticancer agents such as cisplatin. In this study, we conducted virtual screening of Korea Chemical Bank database targeting PARP-1 and identified several potent PARP-1 inhibitors with submicromolar IC_{50} values (77–79 nM). We then examined the chemosensitization of cisplatin by pre-treatment of PARP-1 inhibitors in cisplatin-resistant human gastric cancer cells. Our results show that PARP-1 inhibitors suppress the formation of poly(ADP-ribose) and enhance the cytotoxicity of cisplatin.

Poly(ADP-ribose) polymerase-1 (PARP-1) is one of the most abundant nuclear enzymes in the eukaryote and functions as a DNA damage sensor and signaling molecule binding to both single- and double-stranded DNA breaks. Upon DNA damage, PARP-1 is activated and binds to DNA breaks, which catalyses the poly(-ADP-ribosyl)ation reactions whereby ADP-riboses are transferred from nicotinamide dinucleotide (NAD^+) to glutamic and less commonly to aspartic and lysine residues in PARP-1 itself and their substrates including histones. Accumulation of negative charges on PARP-1 and histones results in a repulsion force and subsequent dissociation of these components from DNA. The resultant chromatin relaxation facilitates for DNA damage repair.[1–4] Binding of PARP-1 to single strand breaks (SSBs) recruits components of DNA damage repair pathways such as X-ray repair cross complementing protein 1 (XRCC1) and protects them from converting into double strand breaks (DSBs).[1,5] In DSBs repair, the role of PARP-1 has been revealed through the identification of PARP-1-dependent alternative non-homologous end joining (NHEJ) pathway.[6–9] However, in response to genotoxic agents, cells express different behaviors dependent on stimulus intensity which triggers PARP-1 activity. PARP-1 activation by mild to moderate genotoxic stimuli facilitates DNA repair. Thus cells survive without the risk of passing mutated genes. More severe DNA damage induces apoptosis in which caspase inactivates PARP-1, subsequently eliminating cells with severe DNA damage. However, over-activation of PARP-1 by excessive DNA damage leads to depletion of NAD^+ and ATP which prevents apoptotic cell death. Under this condition, the inhibition of PARP-1 preserves NAD^+ and ATP, therefore allow cells either to function normally or die via apoptotic pathway.[10] Based on these observations, PARP-1 inhibitors have been suggested in single or combination therapy of various diseases.[11–18]

Formation of cisplatin–DNA adducts which trigger different downstream signaling pathways is the main cause for cytotoxic effect of cisplatin.[19] Interestingly, PARP-1 showed high affinity to the most common 1,2-d(GpG) and this affinity decreases upon automodification which implicates the role of PARP-1 in repair of cisplatin-induced DNA damage.[20,21] Therefore, combination between PARP-1 inhibitor and cisplatin enhances cytotoxicity effect of cisplatin which has been demonstrated by recent studies.[22–29] In this study, we first reported chemosensitizing effect of PARP-1 inhibitors on long-term cisplatin-resistant gastric cancer cells.

PARP-1 consists of three main domains: the N-terminal DNA binding domain, the auto-modification domain and the C-terminal catalytic domains. Most of PARP-1 inhibitors imitate interaction between PARP-1 catalytic domain with its substrate, NAD^+. Early PARP-1 inhibitors were analogues of 3-amino benzamide because it was observed that the benzamide moiety was crucial for the specific binding to the enzymatic site, forming three key hydrogen bonds to the enzyme. From observations that binding affinity would be significantly increased when the carboxamide group, which is normally free to rotate, was restricted into lactam, many classes of PARP-1 inhibitors have been discovered such as amino-ethyl pyrrolo dihydroisoquinolonone, tricyclic quinoxalinone,

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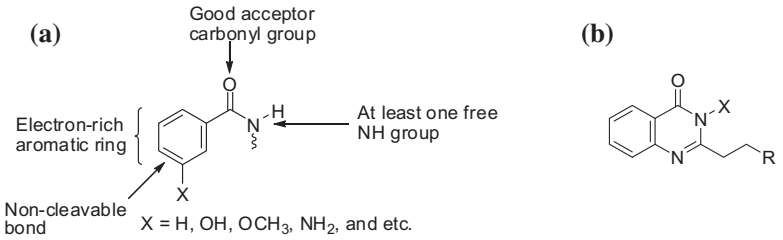


Figure 1. (a) Requirements for PARP-1 inhibitors. (b) Pharmacophore query.

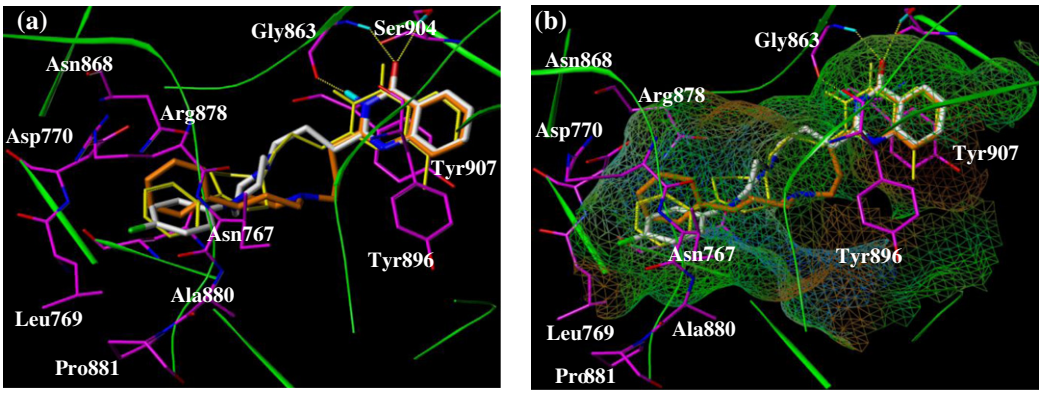
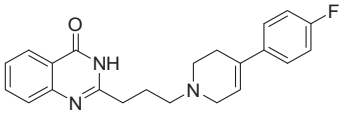
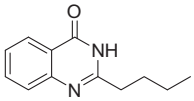
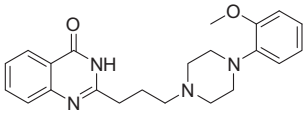
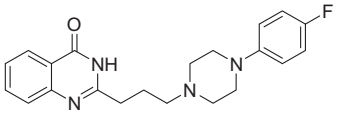
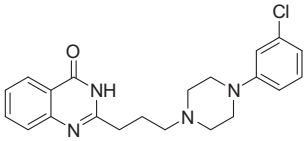
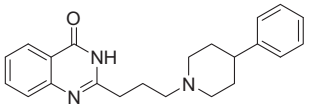
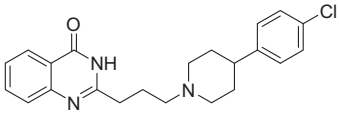


Figure 2. (a) FlexX-docked pose[45] of D31 and D36 into active site of PARP-1. FR257517 (yellow) in the X-ray crystal structure of PARP-1 (PDB ID: 1UK0) are showed for comparison. Hydrogen bonding interaction between D31 (atom type) and D36 (orange) are represented in yellow dotted lines. (b) Lipophilic potential surface map of the catalytic site pocket of PARP-1 is demonstrated in the docking model of D31 and D36. Lipophilicity increases from blue (hydrophilic) to brown (lipophilic).

quinazolinone, phthalazinone, benzimidazole, indole, and isoquinoline derivatives.[35–43] Analysis of different classes of PARP-1 inhibitors had suggested three structural features which must be taken into design of PARP-1 inhibitors: (i) electron-rich aromatic ring, (ii) a non-cleavable bond in the 3 position relative to carboxamide group, (iii) carboxamide moiety which is free to rotate or restricted to ring system.[2] Based on the model, we created a pharmacophore query (Fig. 1b) in which the quinazolinone core makes a sandwiched hydrophobic interaction, including π–π interaction with the phenyl ring of tyrosine residues Tyr907 and CH–π interaction with Cβ of Tyr869, the oxygen of carbonyl group will form hydrogen bonds with Ser904Oγ and Gly863NH whereas NH moiety forms hydrogen bond with Gly863C=O. The side chain consists of at least two carbon units allows the R group to reach the deep pocket located in the auto-modification domain of PARP-1. The pharmacophore-based virtual screening was performed against Korea Chemical Bank (http://www.chembank.org/) chemical database containing about 5 million chemicals using the Unity program in Sybyl 8.1. Only 27 compounds satisfied the pharmacophore query (Fig. 1b), and they were subjected to PARP-1 inhibitory assay.[34] Seven hit compounds were identified and the two most potent compounds showed nanomolar IC_{50} values (Table 1). However, these two compounds, D31 and D36, were registered for patent by KuDo pharmaceutical company for PARP-1 inhibitory activity. The other compounds except D30, have not been patented but they have already been reported in the literatures.[40–43]

Drug resistance is one of the greatest obstacles in cancer therapy. Due to their ability to transform necrotic cell death into apoptotic cell death, PARP-1 inhibitors enhance the effect of different DNA-alkylating agents such as topoisomerase I inhibitors, doxorubicin, cisplatin, oxaliplatin, gemcitabine, etc.[24–31] To examine whether PARP-1 inhibitors enhance the effect of cisplatin in resistant cell lines, we conducted chemosensitizing experiments of PARP-1 inhibitor against cisplatin-resistant cell line. For this experiment, cisplatin-resistant human gastric cancer cell line (YCC-3/D)

Table 1
PARP-1 inhibitors identified from Korean Chemical Bank

ID	Chembank ID	Hit Structure	IC_{50}[44] (μM)
D04	6338MJ0004		0.304
D07	2139SI0007		8.886
D30	6254SO0030		0.189
D31	6255SO0031		0.077
D32	6256SO0032		1.008
D36	6355SO0036		0.079
D38	6357SO0038		0.388