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Synthesis and evaluation of tricyclic derivatives containing a non-aromatic amide as inhibitors of poly(ADP-ribose)polymerase-1 (PARP-1)

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ABSTRACT

Highly potent poly(ADP-ribose)polymerase-1 (PARP-1) inhibitors, including 9-hydroxy-1,2-dihydro-4*H*-thiopyrano[3,4-*c*]quinolin-5(6*H*)-one derivatives with a non-aromatic A-ring, were synthesized. Among the derivatives, **12a** showed low nanomolar enzyme and cellular activity (IC_{50} = 42 nM, ED_{50} = 220 nM) with good water solubility. Further, **12a** exhibited microsomal stability in vitro and brain permeability in vivo.

The poly(ADP-ribose)polymerase (PARP) family is immediately stimulated by DNA damage.[1] When DNA is damaged, PARPs use nicotinamide adenine dinucleotide (NAD^+) as substrate to synthesize a polymer of ADP-ribose on the PARP protein itself and various other protein acceptors, and then carry out DNA repair, recombination, cell proliferation, or cell death and genomic stability.[2] PARP activation has been shown to mediate both ischemic brain injury and cancer by caspase-independent cell death and DNA repair.[3] PARP-1 (EC 2.4.2.30) was the first enzyme of this family to be discovered, and is the most abundant member of the PARP enzyme family in eukaryotes.[2,4] The role of PARP-1 is important in a number of cellular processes, thus it is regarded as a target for treating

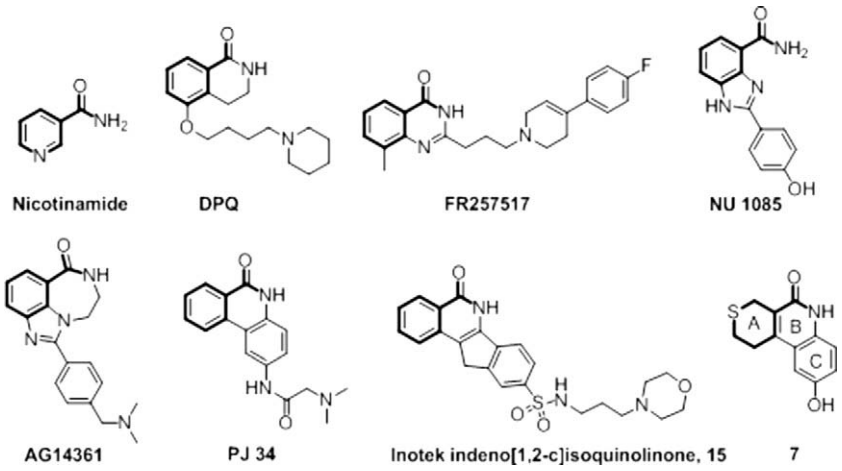

Figure 1. Previously reported PARP-1 inhibitors and compound **7**.

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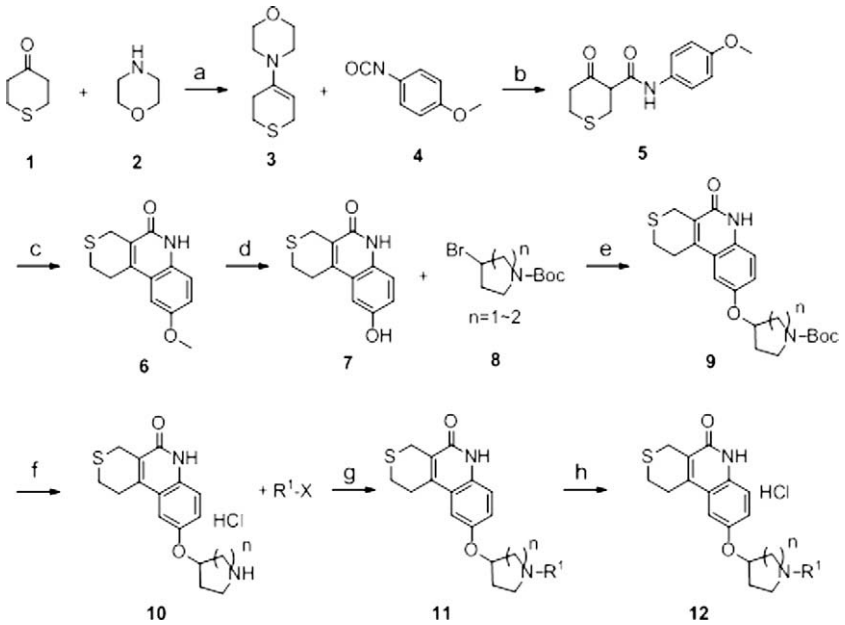


Scheme 1. Reagents and conditions: (a) *p*-TsOH?H_2O, toluene, reflux, 12 h; (b) (i) toluene, 1 h; (ii) 2 N HCl, 24 h; (c) 70% H_2SO_4, 14 h; (d) BBr_3, CH_2Cl_2, 2 h; (e) K_2CO_3 or KOH, *i*-PrOH, 24 h; (f) HCl, 1,4-dioxane, 12 h; (g) K_2CO_3, MeCN, 70 °C; (h) HCl, 1,4-dioxane.

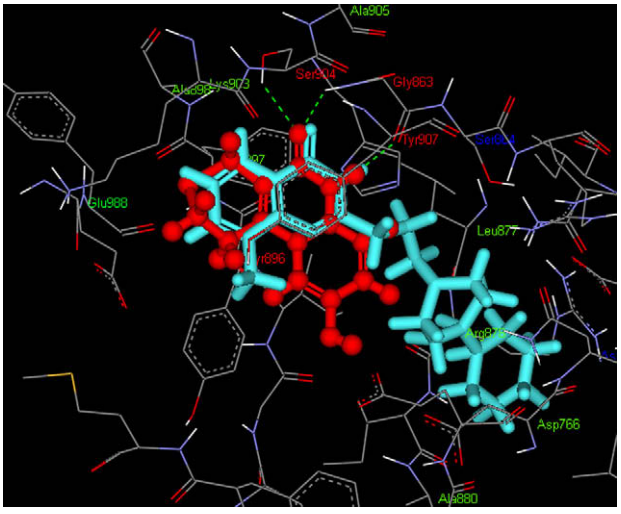


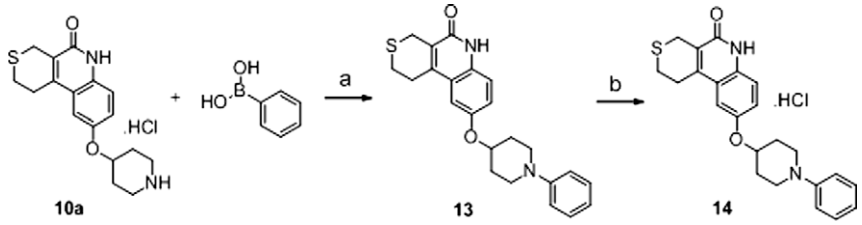
Figure 2. Docking of compound **7** (red color, ball and stick) and FR257517 (blue color, stick)[11] in the catalytic domain of human PARP-1 (PDB code: 1UK0).

diseases related to ischemia-reperfusion injury and cancer. Indeed, PARP-1 inhibitors have been shown to be effective in animal models of ischemic stroke, traumatic brain injury, Parkinson's disease and cancer.[5–7]

Recently, a variety of PARP-1 inhibitors have been reported.[6,8–15] Most of the PARP-1 inhibitors are competitive with NAD^+ and these structures are typically nicotinamide or benzamide analogs (Fig. 1). The aromatic amide group of these compounds form hydrogen bonds with the Gly-863 and Ser-904 of the PARP enzyme and also bind to Tyr907 and Tyr896 of the nicotinamide-ribose binding site (NI site) by a sandwiched hydrophobic interaction.[8,11,14] But, some of the PARP-1 inhibitors, fused uracils, had a non-aromatic amide.[16] On the basis of these compounds, we carried out novel class of PARP-1 inhibitors.

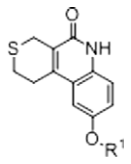
In this Letter, we describe the synthesis, the structure–activity relationship (SAR) study and the biological evaluation of tricyclic compounds that contain non-aromatic amides as potential PARP-1 inhibitors. Crystallographic structure of the human PARP-1 catalytic domain (PDB code: 1UK0) was obtained from the Protein Data Bank (PDB) database,[11] and a docking study was performed to approach the development of potent inhibitors. The result led us to design analogs containing a non-aromatic A-ring. The first compound **7** which included a non-aromatic A-ring, fit the NI site very well even though its conformation was not flat (Fig. 2).

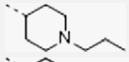
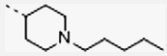
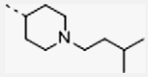
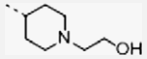
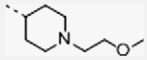
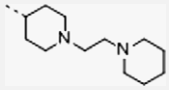
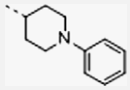
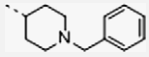
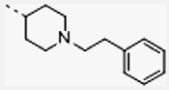
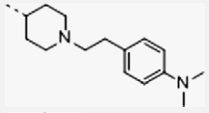
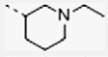
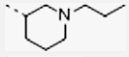
To verify this docking study, tricyclic 1,2-dihydro-4*H*-thiopyrano[3,4-*c*]quinolin-5(6*H*)-one derivatives were synthesized, as outlined in Scheme 1. The 4-thianone **1**, which was synthesized by method in previous report, reacted with a secondary amine by azeotropic distillation with toluene.[17,18] The reaction of the enamine **3** with the isocyanate **4** gave the ketoamide **5**, which was cyclized to the thiopyranoquinolinone **6** in 70% sulfuric acid at room temperature.[19] Demethylation of **6** with Boron tribromide in dichloromethane gave alcohol **7**. Alkylation of **7** with the appropriate bromide **8** in the presence of base led to the Boc-protected amine **9**. Alkyl amine **11** was synthesized by deprotection under acidic condition and coupling reaction under basic condition. Final-



Scheme 2. Reagents: (a) $Cu(OAc)_2$, Et_3N, MeCN; (b) HCl, 1,4-dioxane.

Table 1
Enzyme and cellular activity of the synthesized compounds



Compound	R^1	IC_{50}[a] (μM)	ED_{50}[b] (μM)
15	—	0.040	1.00
7	H	0.099	ND[c]
12a		0.042	0.22
12b		0.045	0.58
12c		0.071	0.74
12d		0.029	1.60
12f		0.040	1.26
12g		0.173	>30
14		0.695	1.86
12h		0.053	0.80
12i		0.038	0.36
12j		>10	9.44
12k		0.028	0.89
12l		0.039	0.58
12m		0.025	0.28
12n		0.056	0.74
12o		0.080	0.73
12q		0.013	0.35

[a] Enzymatic assays followed a commercially available protocol (Trevigen kit, 4671-096-K) in 384-well plates. Values are the mean of triplicate experiments.
[b] The CHO-K1 (Chinese hamster ovary) cell line was used for cell-based assay. Values are the mean of quadruplicate experiments.
[c] ND; not determined.

ly, the N-substituted amine salt **12** was prepared by 3.7 N HCl in 1,4-dioxane to enhance water solubility. In addition to **12**, the synthetic procedure for phenyl amine salt **14** was outlined in Scheme 2. N-Arylation of amine **10a** with phenylboronic acid in the presence of Copper(II) acetate gave phenyl amine **13**, followed by 4.0 N HCl in 1,4-dioxane at room temperature to form **14**.[20]

Recently, the indeno[1,2-*c*]isoquinolinone **15**, reported by Inoteck, showed 1 nM inhibitory activity (Table 1).[13] However, according to our assay protocol, indeno[1,2-*c*]isoquinolinone **15** had an IC_{50} value of 40 nM. The activity of various 1,2-dihydro-4*H*-thiopyrano[3,4-*c*]quinolin-5(6*H*)-one derivatives is summarized in Table 1. When piperidine analogs were fused to the C-9 hydroxy position, hydrophobic interactions with the adenine–ribose binding site (AD site) and water solubility were increased. The *N*-propyl and pentyl piperidine derivatives (**12a**, **12b**) showed good enzyme and cellular activity, and water solubility compared to **7**. Moreover, **12a** was 4.5-fold more active than **15** in the cell-based assay. The *N*-bulky alkyl piperidine derivative **12c** was less active than **12a** due to bumping slightly to the active site. The *N*-(2-hydroxy) and *N*-(2-methoxy)ethyl piperidine derivatives (**12d**, **12f**) retained moderate activity and the *N*-(2-piperidinyl)ethyl piperidine derivative **12g** was twofold less active than **7** in the enzyme assay. However, **12d**, **12f**, and **12g** displayed weaker activity in the cell-based assay. Additionally, *N*-aryl alkyl piperidine derivatives (**12h**, **12i**) demonstrated good potency, but rigid or polar bulky derivatives (**14**, **12j**) displayed a loss of potency. Modification of the nitrogen position (**12k**, **12l**, **12m**, and **12n**) showed good potency, similar to that of **12a**. Reduction of ring size (**12o, 12q**) led to moderate potency and **12q** showed a sixfold improved potency compared to **12o**.

Table 2
Rat PK profile of **12a**[a] (10 mg/kg), $n = 3$

Compound	**12a**
Dose (mg/kg)	10
AUC_{0-inf} (h ng/ml)	2908
CL (l/h/kg)	3.4
$t_{1/2}$ (h)	3.45

[a] Values were detected by LC/MS/MS after intravenous administration.

Table 3
Brain/plasma concentrations of **12a** in the rat[a] (10 mg/kg), $n = 3$

Time (h)	Brain (ng/g)	Plasma (ng/ml)	Ratio
0.17	1705	2480	0.69
0.75	391	610	0.64
4.00	13	18	0.72

[a] The concentration was detected by LC/MS/MS.

Table 4
Human liver microsomal stability of **12a**[a]

Drug	$t_{1/2}$ (min)
Buspirone	3.8
12a	52.6

[a] Microsomal activity was detected at 0, 15, 45, and 80 min by LC/MS/MS. The incubation temperature was 37 °C. Values are the mean of triplicate experiments. Buspirone was used as reference.

We examined pharmacokinetic (PK) characteristics, brain/plasma concentration, and microsomal stability of **12a**. In the PK studies, **12a** showed a relatively long intravenous half-life and high AUC concentration (Table 2). The concentration of **12a** in plasma and brain was detected by LC/MS/MS after intravenous injection in rats. The brain/plasma ratio was 0.64–0.72 over four hours (Table 3). In the human liver microsomal stability test, **12a** showed good metabolic stability with a 52.6 min half-life (Table 4).[21]

In conclusion, we report the synthesis and biological evaluation of tricyclic derivatives with a non-aromatic amide as potent PARP-1 inhibitors. Compound **12a** was found to be highly potent in enzyme and cell-based assays ($IC_{50} = 42$ nM, $ED_{50} = 220$ nM) with good water solubility and brain penetration. These findings suggest that the PARP-1 inhibitor **12a** could be a useful therapeutic candidate for ischemic stroke and cancers. Further evaluation of this class of derivatives is ongoing and will be reported in the near future.

Acknowledgments

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