Discovery of Quinazolinedione Derivatives as Potent PARP1 Inhibitors

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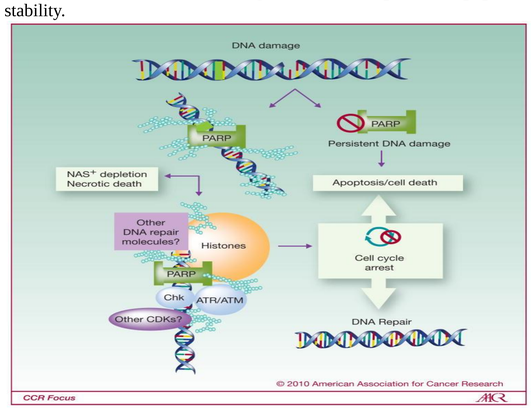
Introduction

Poly(ADP-ribose) polymerases 1 (PARP1) is the most abundant and well characterized protein of PARP family members, catalyzing the polymerization of poly(ADP-ribose) on target proteins. PARP1 participates in a variety of cellular functions, including chromosome stability, signal transduction and the regulation of gene transcription. One of key roles of PARP1 is to repair DNA damage ensuring genomic stability.



Adapted from Annunziata CM,et al. Clin Cancer Res. 2010,16(18):4517

Inhibiting PARP1 showed anti-tumor activity by preventing damaged DNA repair in preclinical models and clinical trials. PARP1 inhibitors have been used as monotherapy in patients with BRCA1/2 deficient cancers and as combination therapy with DNA-damaging chemo-therapeutic agents in patients with advanced solid tumors. Promising compounds including AstraZeneca' Olaparib (AZD-2281) and Abbvie's Veliparib (ABT-888) exhibited high response rate and good tolerability in clinical trials.

We discovered a series of quinazolinedione derivatives as novel PARP1 inhibitors with outstanding in vitro and in vivo efficacy .

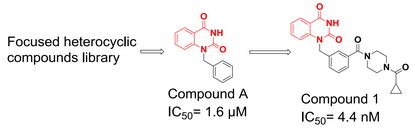
Materials and Methods

PARP enzymatic assay. HT Universal PARP Assay Kit (Trevigen) was used to measure the incorporation of biotinylated poly(ADP-ribose) onto histone proteins. IC50 values of PARP inhibitors were determined using Graphpad prism software.

Cell proliferation assay. The effect of PARP inhibitors on the cell survival of breast cancer cell line MDA-MB-436 were measured by CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega).

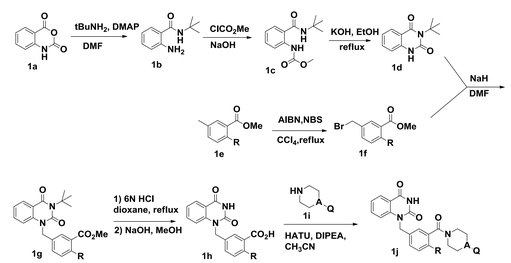
Xenograft model. Antitumor activity of PARP inhibitors was evaluated in BALB/cA-nude mice bearing SW620 colorectal cancer cells. Briefly, 5x105 SW620 cells were inoculated subcutaneously into mice. Tumor-bearing mice were administrated with PARP inhibitors and TMZ for 42 days. Tumor volume and body weight were measured twice a week.

Discovery of Quinazolinedione Lead

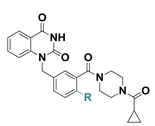


We designed a focused library with different heterocyclic cores to mimic key hydrogen bonding network in nicotinamide binding pocket of PARP1 enzyme. Through screening for PARP activity in enzymatic assay, we identified Compound A with moderate potency. Addition of a piperazine amide side chain led to compound 1 with significantly improved (>350fold) potency in enzymatic assay.

General Synthetic Route



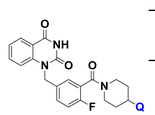
Modifications on Middle Benzene Ring



Compound#	R	PARP-1 IC_{50}(nM)	MDA-MB-436 GI_{50}(μM)
1	H	4.4	4
2	F	2.1	0.086
3	Cl	9.8	2.2
4	CF_3	313	NA

➢ 2-F substitution significantly improved cell potency

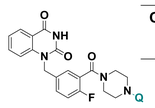
SAR of 4-substituted Piperidines



Compound#	Q	PARP-1 IC_{50}(nM)	MDA-MB-436 GI_{50}(μM)
5	F	12	5
6	-OH	4.5	1.3
7	-OEt	3.8	0.019
8		2.7	0.008
9		15	3.4
10		3.4	1
11		3.5	0.2

➢ O linker analogs showed excellent cell potencies

SAR of 4-substituted Piperazines



Compound#	Q	PARP1 IC_{50}(nM)	MDA-MB-436 GI_{50}(nM)
12		1	63
13		0.9	81
14		0.7	42
15		2.3	19
16		1.5	12
17		1	12
18		0.6	8
19		1.1	3

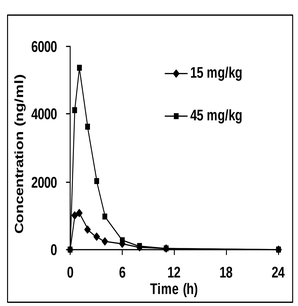
➢ Compound 18 exhibited excellent in vitro potency

In Vitro profile of Compound 18

HLM t1/2	Solubility (HSO_4 salt)	HERG	CYP450 (1A2, 2C8, 2C9, 3A4)
15 min	>17 mg/mL	>10 μM	>20 μM

➢ Relatively high clearance in human liver microsomes
➢ Excellent water solubility
➢ No CYP or HERG inhibition issues

Rat PK of Compound 18

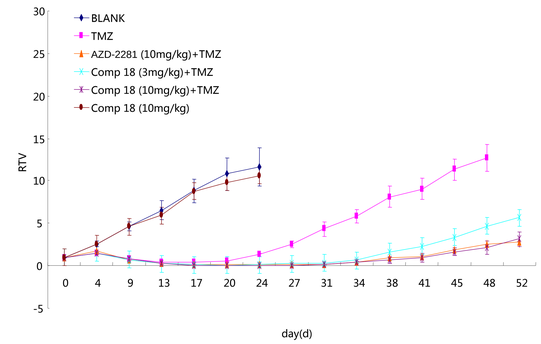


Dose(mg/kg)	15	45
Cmax(ng/ml)	1099	5696
AUC (h*ng/ml*h)	3239	14209
T1/2(h)	1.3	1.2
CL(L/h/kg)	19	33
Vdss(L/kg)	20	22
F	26%	

Time course of Compound18 plasma concentration in rat following a single oral dose. PK parameters for oral dose were listed in the table.

In Vivo Efficacy

Efficacy of Compound 18 in SW620 xenograft model (oral administration).



➢ Compound 18 in combination with Temozolomide (TMZ) showed dose-dependent inhibition of tumor growth in SW620 xenograft model following oral administration.

➢ The potency of Compound 18 was comparable to AZD 2281 at the same dose level.

CONCLUSION

➢ We have discovered a novel series of quinazolinedione compounds as potent PARP1 inhibitors. From SAR study we identified Compound 18 as the leading candidate.

➢ Compound 18 exhibited excellent in vitro potency (~4-fold more potent than AZD-2281).

➢ Compound 18 has good oral bioavailability and PK profile.

➢ Compound 18 showed excellent in vivo efficacy in SW-620 xenograft mice model. It dramatically shrank tumor growth when combined with TMZ.

➢ Compound 18 has the potential to be an excellent clinical candidate as PARP1 inhibitor.