Design and Development of Piperazinediones as Antitumor Agents

王惠珀

台北醫學大學藥學院

簡介

Collaboration between CGU and NTU lead to the innovation of TW01 NCEs as antitumor agents (Fig. 1a). Technical transfer to AngioRx Inc. lead to the grant of IPR in several nations (Table 1). Chemical modification of TW01 resulted in the generation of TW01001 and TW01002 as new leads (Fig. 1b).

Table 1. IPR status of TW01 series analogues.

Status	countries
Issued	US, Singapore, New Zealand, Russia, South Africa, Malaysia
Published	Brazil, China, Europe, Hungary, Indonesia, Korea
Pending	Australia, Canada, Czech, India, Japan, ROC
CIP	US, 2004

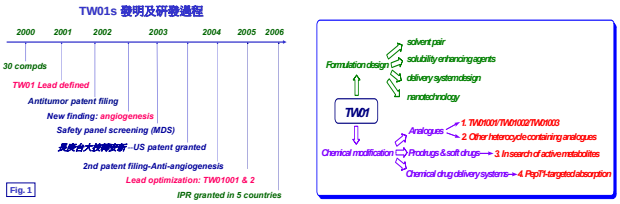


Fig.1. (a) The discovery chronology of TW01 analogues and (b) the generation of TW01001 & TW01002 as the leads.

成果

I. Discovery of NCE

TW01 analogues exhibited anticancer (IC_{50} ~10^{-8} M, and anti-angiogenesis activities (Fig. 2). The lead TW01 demonstrated (1) inhibition on 18 cancer-related serine/threonine kinases (IC_{50}: 1.73 μM/PKBa/Akt1, 2.32 μM/Fyn, 1.4 μM/Erk & 11.1 μM/MEK), PI 3 kinases, abl tyrosine kinase and tyrosine phosphatase (IC_{50} 1 ~ 0.1 μM); (2) the mechanism of action different from taxol by inhibiting microtubule polymerization; (3) higher inhibitory activity than taxol on drug-resistant cancer cell lines (Table 2); (4) over 30 of the increase of life span in mice bearing A549 human non-small cell lung cancer or HA22T human hepatoma (Fig. 3a & 3b); (5) wide safety margin as screened on 136 proteins including 118 receptors, 10 transporters, 7 carrier proteins and 15 CYP450 metabolic enzymes (Fig. 4).

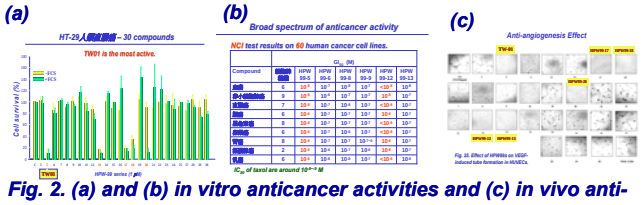
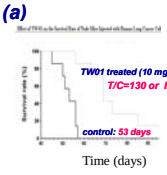


Fig. 2. (a) and (b) in vitro anticancer activities and (c) in vivo anti-angiogenesis effect of TW01 analogues.

Cell line	IC_{50} (M) TW01	IC_{50} (M) Taxol	Potency ratio TW01/Taxol
Colorectal cancer HCT-15	$4.0x10^{-8}$	$2.1x10^{-7}$	5.2
Renal cancer ACHN	$9.7x10^{-8}$	$1.5x10^{-6}$	15.4
CAKI-1	$6.3x10^{-8}$	$2.0x10^{-7}$	3.1
TK-10	$3.8x10^{-8}$	$6.9x10^{-8}$	1.8
UO-31	$5.1x10^{-8}$	$1.0x10^{-7}$	19.6
Breast cancer MCF7/ADR-RE	$2.6x10^{-8}$	$3.1x10^{-6}$	119.2

Table 2. Cytotoxicity of TW01 and taxol on drug-resistant cancer cell lines.



	30%存活天數(n=10)
控制組	7d天
Doxorubicin	8d天
TW01 (3 mg/kg)	7d天
TW01 (10 mg/kg)	7d天
TW01 (30 mg/kg)	22d天

Fig. 3. (a) Increase of life span and (b) long term survival of mice bearing HA22T after treatment with TW01.

Treatment	#370300, HT-29 Colon (μM) IC_{50}	TGI	LC_{50}	#370400, A-498 Kidney (μM) IC_{50}	TGI	LC_{50}	# 371000, A549, Lung (μM) IC_{50}	TGI	LC_{50}
TW01	0.11	5.3	>100	0.48	>100	>100	0.083	>100	>100
HPW99-6	2.0	>100	>100	>100	>100	>100	14	>100	>100
HPW99-12	2.0	>100	>100	7.9	>100	>100	2.6	>100	>100
HPW99-13	2.2	12	67	11	62	>100	9.8	>100	>100
HPW99-17	0.028	2.9	>100	0.21	50	>100	0.021	>100	>100
HPW99-18	7.8	21	57	1.7	30	>100	8.2	>100	>100
HPW99-20	7.6	>100	>100	4.3	40	>100	14	>100	>100
Mitomycin	0.085	1.8	>10	0.21	0.65	2.0	0.047	0.34	2.5

Fig. 4. (a) Wide safety margin of TW01 analogues and (b) selectitve toxicity of TW01.

II. Chemical modification for lead optimization

Although TW01001 and TW01002 are 5-10 times less active than TW01 against cancer cell lines, both analogues demonstrated higher degree of safety in MTD studies (Fig. 5). With improved systemic bioavailability ($t_{1/2}$, 230 and 343 times higher fraction of absorption, Fig. 6 & Table 3), higher anti-angiogenesis activities were also demonstrated (10 mg/kg,Table 4 & Fig. 7) ◦ A preliminary anti-tumor study indicated that the time for tumor volume to reach 1200 m^3 was 56% longer in TW01001 (20 mg/kg) treated than in control mice bearing HT29 colon cancer.

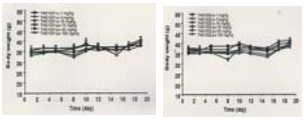


Fig. 5. Acute toxicity of TW01001 and TW01002.

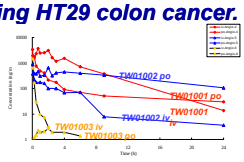


Fig. 6. Plasma concentration time curve of oral and iv. TW01001, TW01002 & TW01003 (n=3).

Table 3. Pharmacokinetic parameters of leads in rats.

	parameter	AUC0-t ng/ml.h	AUC0-∞ ng/ml.h	Cmax ng/ml	T_{max} h	$T_{1/2}$ (h)	CL L/kg.h	F (%)
iv	TW01001	2730.4	3121.1	4522.07		3.74±1.14	0.64	--
	TW01002	855.5	922.7	1099.12		5.24±3.49	2.60	--
	TW01003	219.4	222.1	2151.73		0.51	9.86	--
po	TW01001	14740.7	15479.6	4170.91	0.83	9.12±5.86	2.17	49.60
	TW01002	6555.9	7340.7	680.59	2.67	15.5±12.57	4.63	79.56
	TW01003	5.1	n.d.	1.81	0.83	n.d.	2835.39	0.23

Table 4. In vitro & in vivo anti-angiogenesis effects of the leads.

	HUVEC		In vivo anti-angiogenesis**		
	GI50(uM)	Potency ratio	3 mg/kg	10 mg/kg	30 mg/kg
Taxol	0.013	4	--	--	--
TW01	0.055	1	45%	10%	5%
TW01001	0.10	1/1.8	34%	<1%	<1%
TW01002	0.25	1/4.5	4%	<1%	<1%
TW01003	0.12	1/2.1	<1%	<1%	<1%

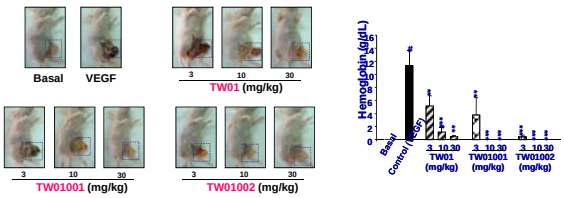


Fig. 7. Anti-angiogenesis effect of TW01001, TW01002 and TW01003.

結論

1.TW01001 and TW01002, modified from TW01 with improved PK profile demonstrated anti-angiogenesis and antitumor activities, are considered the lead NCEs for prelinical development.

2. Formulation of TW01001 and TW01002 for PD/PK optimization need to be conducted for further development of the lead as potential drug candidates.