In Search of Active Metabolites of TW01 as Antitumor and Antiangiogenesis Agents

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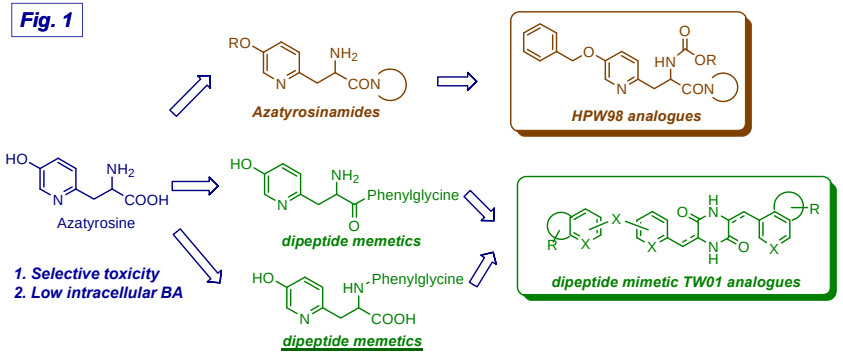
TW01 Analogues as Antitumor and Antiangiogenesis Agents

Patent	*countries*
Granted (NCE, method,antitumor)	*1. US Patent no. 6,635,649 B2, 2002* *2. South Africa patent no. 20029917, 2004* *3. Singapore Patent no. 92962, 2005* *4. New Zealand Patent no. 522680, 2005* *5. Russia Federiation Patent no. 2269520, 2006* *6. Australia Std Patent no. 2001294505, 2006* *7. Advanced Notice, India, 2007.* *8. Advanced Notice - Eurpean Union, 2008.*
Continuation-in-Part (anti-angiogenesis)	*US Patent no. 7,288,545 B2, 2007*
Published	*Brazil, China, Europe, Hungary, Indonesia, Korea, Malaysia*
Pending	*Canada, Czech, India, Japan, ROC*

Transporter approach to optimize PD/PK of Azatyrosine

Fig. 1



Piperazinediones

IC_{50} 10^{-3} M → IC_{50} 10^{-5} M → IC_{50} 10^{-8} M

Table 1

Competitiveness: In vitro activity of TW01s

TW01: Broad spectrum of anticancer activity

NCI test results on 60 human cancer cell lines.

Compound	細胞株種類	TW01 HPW 99-5	HPW 99-6	HPW 99-8	HPW 99-9	HPW 99-12	HPW 99-13
		GI_{50} (M)					
血癌	6	10^{-8}	10^{-7}	10^{-8}	10^{-7}	$<10^{-8}$	10^{-8}
非小細胞肺癌	9	10^{-8}	10^{-6}	10^{-7}	10^{-7}	10^{-8}	10^{-7}
直腸癌	7	10^{-8}	10^{-7}	10^{-8}	10^{-7}	$<10^{-8}$	10^{-7}
腦癌	6	10^{-8}	10^{-7}	10^{-7}	10^{-7}	10^{-8}	10^{-7}
黑色素癌	8	10^{-8}	10^{-7}	10^{-7}	10^{-7}	$<10^{-8}$	10^{-7}
卵巢癌	6	10^{-8}	10^{-7}	10^{-6}	10^{-7}	$<10^{-8}$	10^{-7}
腎癌	8	10^{-8}	10^{-7}	10^{-7}	10^{-7-5}	10^{-8}	10^{-7}
攝護腺癌	2	10^{-8}	10^{-6}	10^{-7}	10^{-6}	10^{-8}	10^{-7}
乳癌	6	10^{-8}	10^{-8}	10^{-8}	10^{-7}	$<10^{-8}$	10^{-8}

IC_{50} of taxol are around 10^{-8-9} M

Data from NCI

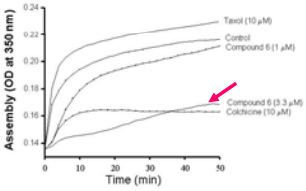


Fig. 2. Cochicine-like mechanism

Table 2

TW01對抗藥性人類癌細胞的抑制活性比taxol強

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

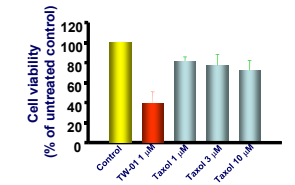


Fig. 3. TW01 & taxol on growth inhibition of PC-3 prostate CA

台大鄭哲明教授

Competitiveness

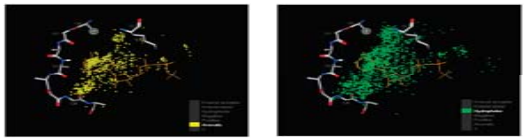
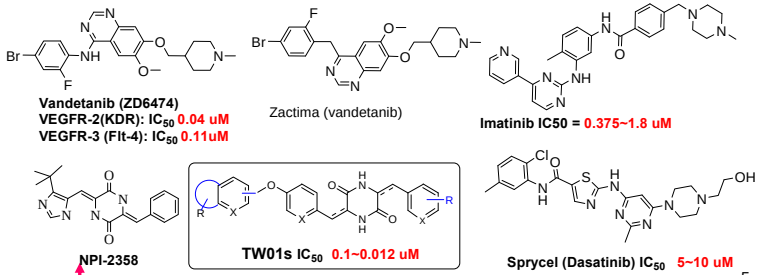


Fig. 4. Pharmacophore mapping of 220 protein kinase inhibitors. J Chem Inf Model, 2007.

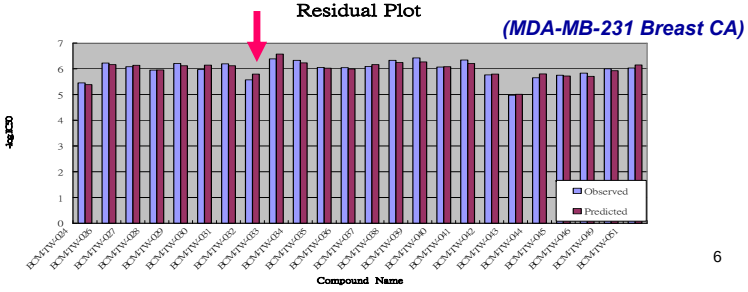


vascular disrupting NPI2358 + docetaxel : Trial In NSCLC, 2008.

Structure-based 4D-QSAR for Activity Pprediction

*Eq : -log IC50 = 5.89 + 3.74*GCOD(3,-4,-1,np) + -1.79*GCOD(2,-3,0,np)*
*+1.30*GCOD(1,6,2,np) + -1.18*GCOD(2,2,2,any) + 0.29*GCOD(2,-4,-1,np)*
N=24 R2 = 0.906 Q2 = 0.818 LOF = 0.028
IPE descriptors: np—non polar, p+: (+)polar atom, p-: (-)polar atom.

Fig. 5. Reliability of the model : Difference between predicted and observed response of BCM-TW-032, with largest difference, is (–logIC50) < 0.23 (3~4%).



Structure-based Prediction of Activity (MDA-MB-231 Breast CA)

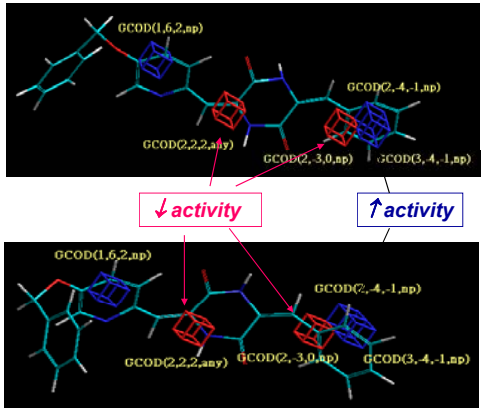


Fig. 6. 4D-QSAR for activity prediction

Predicted active:
–log IC_{50} = 7.19

Predicted inactive
–log IC50 = 4.10

台大生醫電子資訊
曾宇鳳副教授

Mechanism of Action of TW01

Table 3. Inhibition of TW01 on 18 cancer related kinases (Data from MDS Lab.)

Kinases	IC_{50} (/M)
Tyrosine Kinase, Abl (chronic myeloid leukemia related)	0.78
Tyrosine Kinase, Fyn (metastasis related)	2.32
Tyrosine Kinase, Insulin receptor	5.9
Tyrosine Kinase, $pp60^{SRC}$	10
Serine/Threonine Kinase,PKBα/Akt1(cell survival)	1.73
Serine/Threonine Kinase, MEK1 (cancer proliferation related)	11.1
Serine/Threonine Kinase, Erk1 (cancer proliferation related)	1.4
Serine/Threonine Kinases, Erk2, PKC-α, β_I, β_{II}, γ, δ, ε, η, μ, ζ, cdk2/cyclin A	> 10

Table 4. Comparison between TW01 and Gleevec

	TW01*	Gleevec
Tyrosine Kinase, Abl	0.78 μM	0.25 μM**
K562 Cronic Myeloid Leukemia	0.03 μM	0.47±0.04 μM***

**MDS Data. **Buchdunger, Biochim Biophys Acta, 2001. ***Gottschalk, Clin Cancer Res, 2004.*

In vivo activity of TW-01 on HA22T human hepatoma.

Increase of life span (T-C)/C > 55 (30 mg/kg)

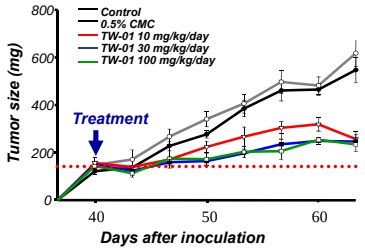
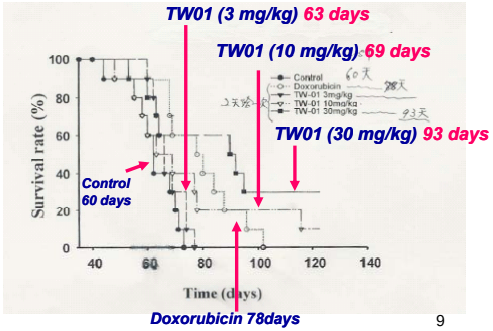


Fig. 7. Inhibition of tumor growth.

Fig. 8. Increase of life span



Data from 台大顧記華教授

Unanswered question: Poor PK with significant PD.
➡ poor absorption or extensive metabolism?

PD/PK Optimization : ***Formulation Becomes the Bottle Neck***

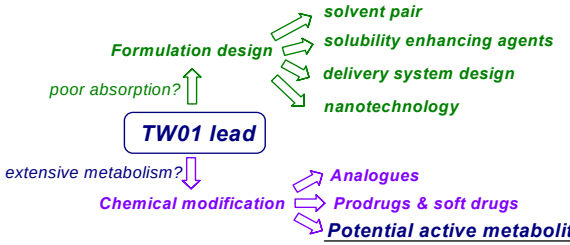


Table 5. PK profile of TW01

	AUC_{0-t} hr*ug/mL	AUC0-∞ hr*ug/mL	Cmax ug/ml	Tmax h	T1/2 h	CL L/hr/kg	F (%)
iv	1.08±0.55	1.13±0.56	1.58±0.67	0	2.34±1.31	0.77±0.37	--
po	0.45±0.13	0.52 ± 0.14	0.18±0.09	1.06±0.33	2.70±1.54	0.6±0.36	1.14±0.61

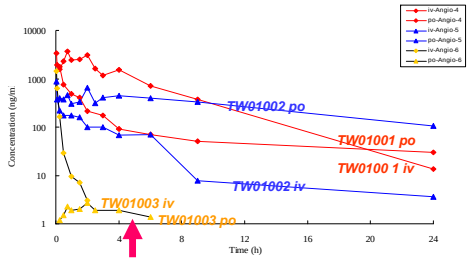


Fig. 9. Most active metabolite of TW01 while not feasible for development as a drug.

Table 6. Comparison of PK profiles (data from contracted Rossetta Co.)

	compds	AUC_{0-t} hr*ug/mL	$AUC_{0-\infty}$ hr*ug/mL	C_{max} ug/ml	T_{max} h	$T_{1/2}$ h	CL L/hr/kg	F (%)
iv	TW01	1.08±0.55	1.13±0.56	1.58±0.67	0	2.34±1.31	0.77±0.37	--
	TW01001	2.66±0.47	3.05±0.24	3.35±1.52	0	3.74±1.14	0.66±0.05	--
	TW01002	0.83±0.45	0.90±0.37	0.87±0.28	0	5.24±3.49	2.55±1.25	--
	TW01003	0.18±0.06	0.19±0.06	1.46±0.99	0	0.53±0.21	11.48±3.21	--
po	TW01	0.45±0.13	0.52 ± 0.14	0.18±0.09	1.06±0.33	2.70±1.54	0.6±0.36	1.14±0.61
	TW01001	14.02±14.05	14.76±13.61	4.17±3.41	0.83±0.63	9.12±5.89	2.31±1.71	46.8±40.0
	TW01002	6.56±5.70	7.28±6.84	0.68±0.34	2.67±1.15	12.42±15.29	4.84±3.71	78.9±51.0
	TW01003	0.01±0.01	0.07±0.01	0.00±0.00	3.00±1.41	1.94±3.37	337.89±58.52	0.76

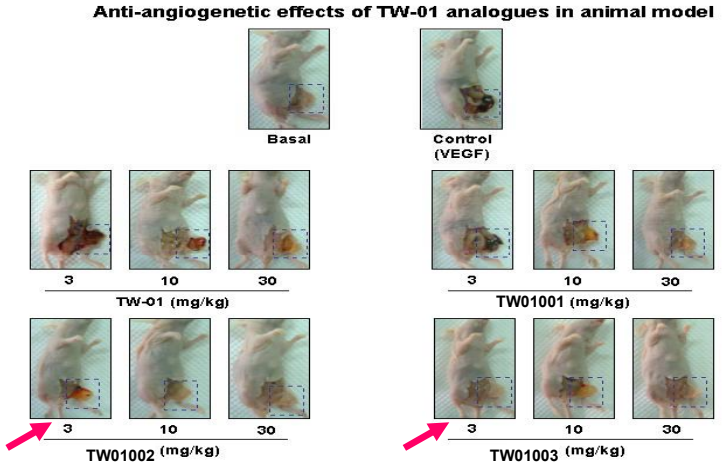
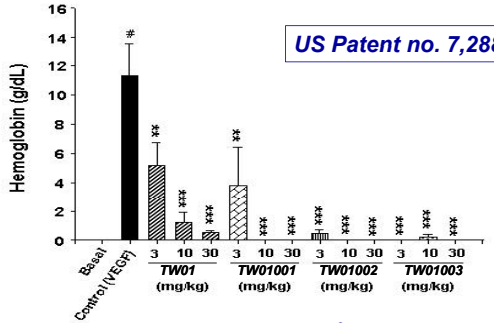


Fig. 10. Nude mice were given s.c. of 500 μl of Matrigel at 4℃ containing VEGF or bFGF (150 ng/ml). Test sample was administered orally once daily. After 6 days, the animals were euthanatized and the plugs were clipped for measurement of angiogenic effects.

Anti-angiogenetic effects of TW-01 analogues in animal model



US Patent no. 7,288,545 B2, 2007

Data from Guh

Table 7. Oral bioavailability and anti-angiogenesis of potential TW01 metabolites.

	Solubility ug/mL	Oral BA*% (n=3)	HUVEC		In vivo anti-angiogenesis**		
			GI50	Potency	3 mg/kg	10	30
Taxol	~40		0.013	4	--	--	--
TW01	23	<1	0.055	1	45%	10%	5%
TW01001	137± 6.26	46.8±40.0	0.100	1/1.8	34%	<1%	<1%
TW01002	286.4±1.2	78.9±51.0	0.250	1/4.5	4%	<1%	<1%
TW01003	197.6 ±0.6	1.72 (n=1)	0.120	1/2.1	<1%	2%	<1%

In search of active metabolites of TW01003

Iv (7 mg/kg) **Oral (36 mg/kg)**

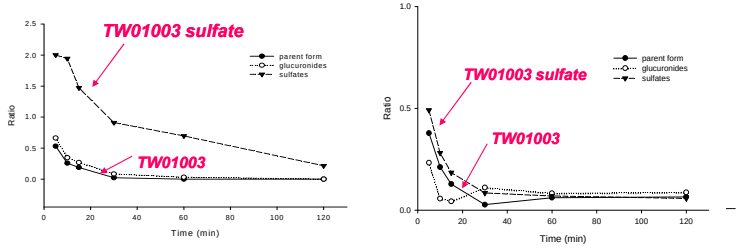
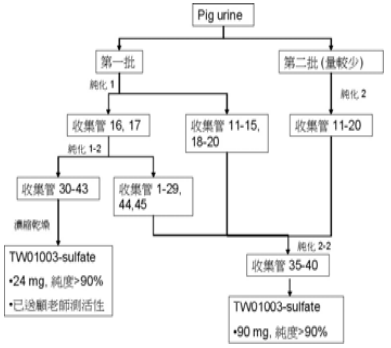


Fig. 10. *Plasma concentration-time curve indicated that the anti-angiogenesis effect of TW01003 might come from its active metabolites. The preliminary Data are from studies in one rat（李珮瑞教授）*

In Search of Active Metabolites of TW01003



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Fast biotransformation of TW01003 to sulfate conjugate

Isolation of the metabolite from pigs is in progress.

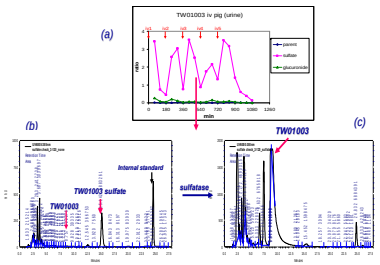

Fig. 7. (a) Urine profile after 5 TW01003 dosing to pig (200 mg/dose eq. to 6.6 mg/kg of 30 kg pig); (b) HPLC of the urine sample 480 min after dosing and (c) urine after sulfatase treatment. Dosing of TW01003 to the pig was conducted in ATIT. 23

台灣動科中心Dr.王耀鴻 Dr.呂彥禮(長庚)

Dr. 王惠珀

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Summary PD/PK Assessment: TW01002 & TW01003 are the choices

1. TW01003 was proved as the active metabolite of TW01.
2. Unsatisfactory PK of TW01003, due to extensive metabolism (clearance), limited its potential to be developed as a drug.
3. TW01 might act as the prodrug of TW01003, if formulation for PK optimization can be achieved.
4. TW01002 and TW01003 designed for retarding the first pass effect (oral BA 47% and 79% respectively), are considered superior to TW01 and TW01003 for further development.

Table 8: Summary PD/PK Assessment: TW01002 & TW01003 are the choices

	Cytotoxicity	Anti-ngio genesis	Water solubility	Oral BA	absorption	metabolism
TW01	++++	++	+	+	+	++
TW01001	+++	+++	++++	++++	++++	+
TW01002	+++	+++	++++	++++	++++	+
TW01003	+++	++++	++	+	+	++++

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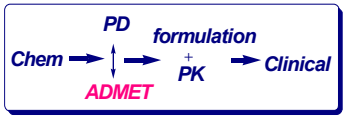
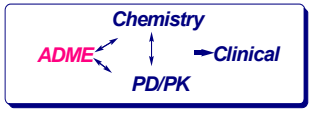
Conclusion: ADME Determined the Success of Drug R & D

Scenario of Drug R & D

PD/PK abreast
PK as passive role
Successful rate: < 1/2000
Time to success > 8 years



Global trend:
Target-well-defined Me-Too
IPR protection limited
6-8 years



My approach:
ADME-driven design for
PD/PK optimization

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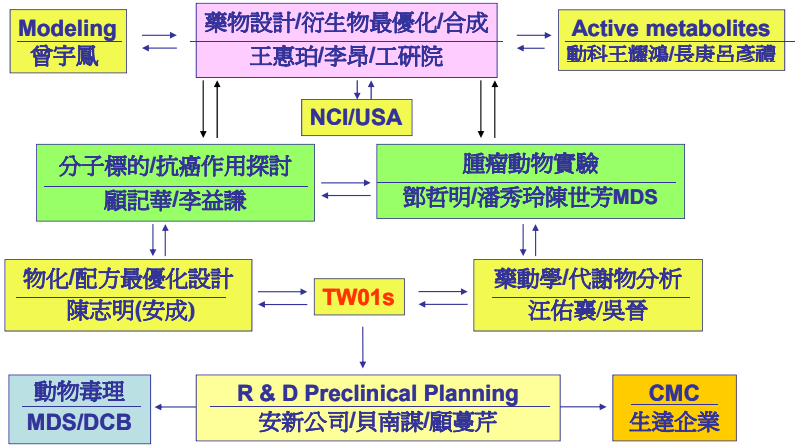
研發團隊



95年度生技製藥國家型計畫: NSC95,96 & 97

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敬請指教!

~Shel Silverstein~

THANK YOU

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