ADME-driven NME Design

~ The development of HPW98s and TW01 as antitumor agents ~

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R & D on target-well-defined NME is not my game.

Active-site directed drug design (in vitro studies):

- ***Too luxurious to play big pharma's game ~ 以卵擊石 ~***
- ***Too precisely designed happy encounter ~ 送作堆 ~***
- ***Ends up with mis-targeting ~ 小時了了大未必佳 ~***
- ***Ends up with multi-targeting ~ 到處留情 ~***
- ***Pearl lost in horizon ~ 遺珠之憾 ~***

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Selective cytotoxicity of azatyrosinamides against *ras*-transformed NIH 3T3 cells

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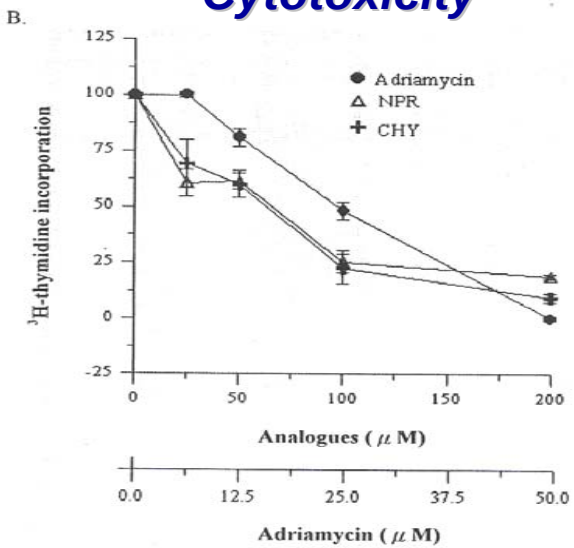
Abstract—This study aims to develop novel azatyrosinamide compounds structurally modified from *ras*-specific antioncogenic azatyrosine. Analogues **4**–**15** were prepared and their inhibition on the growth of wild-type and *ras*-transformed NIH 3T3 cell lines was compared. Compound **12** was found to be the most active with IC_{50} 16.5 ± 2.2 μM which is 458-fold more potent than that of azatyrosine. The selective toxicity, defined as $IC_{50\ \text{wild-type}}/IC_{50\ ras\text{-transformed}}$ for this compound was 138.5.

Story of HPW98-1:

The in vitro test results might not be disappointing.

Cytotoxicity



tumor size

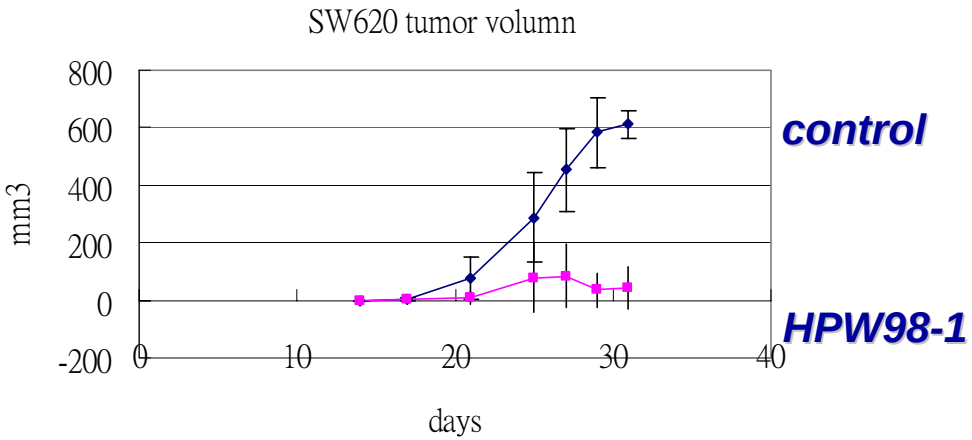
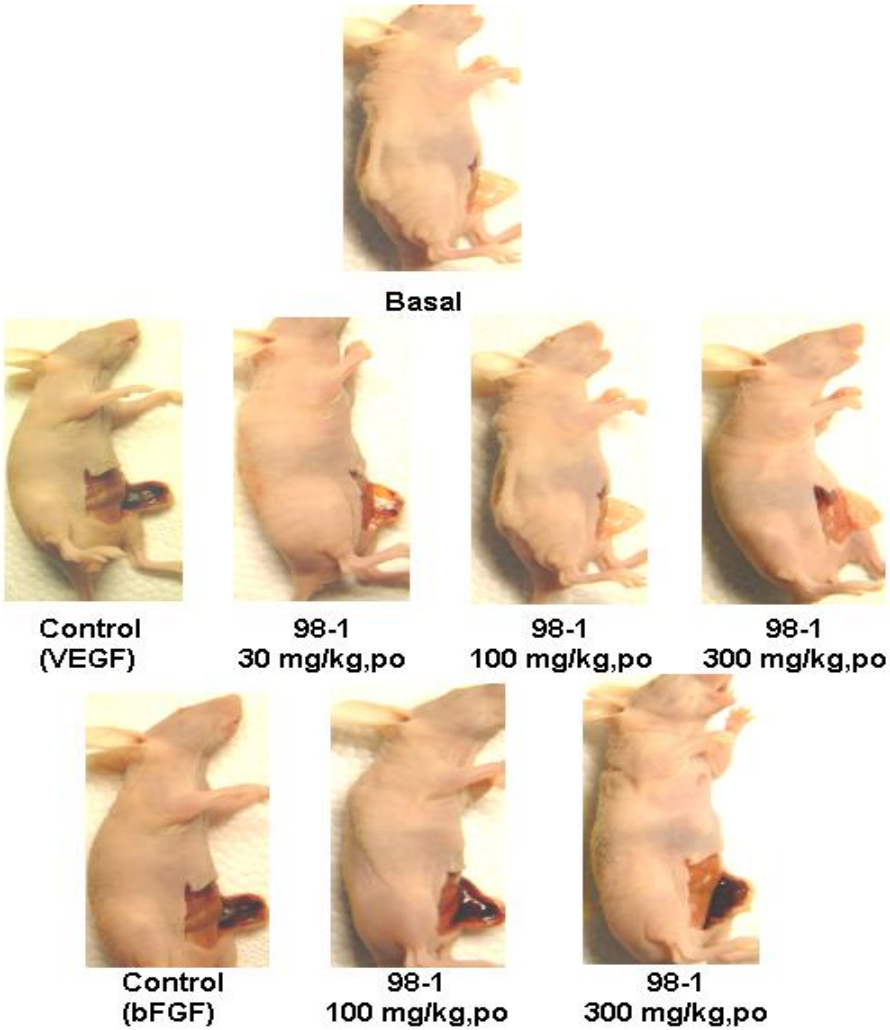


Fig. 1. Average tumor size 20 days after HPW98-1 treatment (oral, 25 mg/kg/daily) was 7% to the control in SCID mice bearing SW620 Human colon. No tumor was observed in 5/10 mice.

~HP Wang etal US patent & PCT 1999~

Anti-angiogenesis turned out to be the mode of action.

Wang etal, US patent 1999 & PCT 2001.



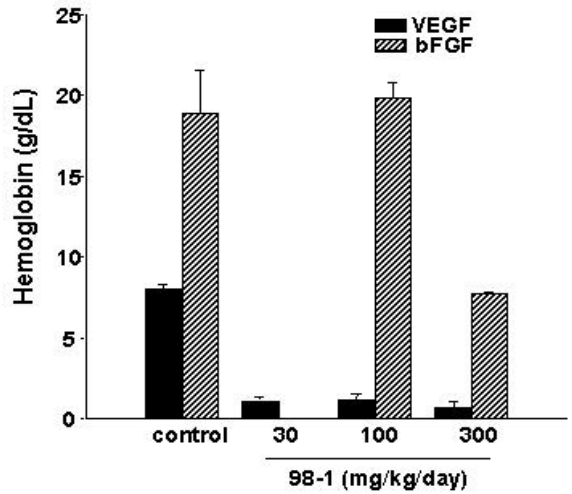


Fig 2. Hemoglobin count after HPW98-1 treatment for 14 days.

Data from Dr. 顧記華

Strategy: How to KISS in Taiwan

IF innovative NME is a must :

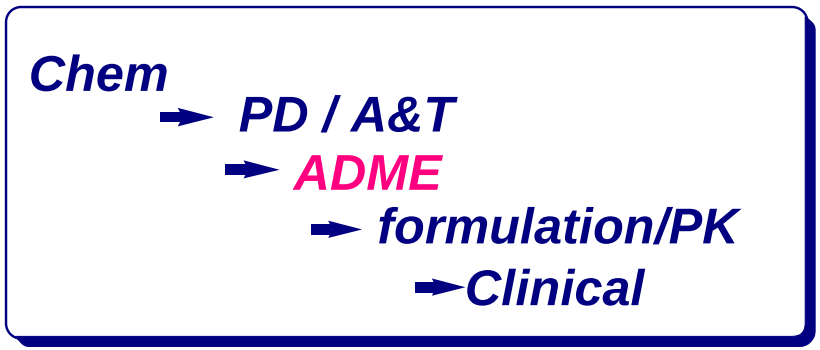
- ***Global aspect ⇒ shoot for high successful rate***
- ***Methodology aspect: quick and dirty***

  ⇒ Chemistry/PD/PK/ADME abreast
- ***How to be pragmatic?***

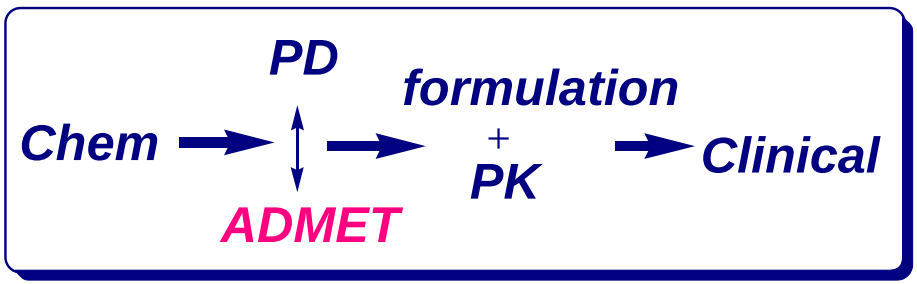
  ⇒ Studies reflect reality

  ⇒ ADME is full of mechanism for drug design.

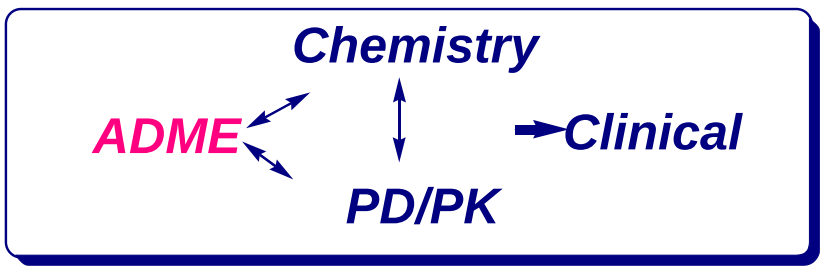
Scenario of Drug R & D



PD/PK sequential -- 串聯式出場
PK as passive & supporting actor
Successful rate: < 1/2000
> 8 years



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



Supporting actor
becomes director

Theme: Amino Acid drugs are my kids. D-Phenylglycine is my true love.

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1. Theory building:

α-methyldopa $\Rightarrow$ AA-α-methyldopa

2. Application:

Dopa $\Rightarrow$ AA-AA-Dopa *(ROC/US patent 1997)*

3. Azatyrosine $\Rightarrow$ AA-AA-Azatyrosine *(not applicable)*

4. AA-Azatyrosine $\Rightarrow$ HPW98s *(ROC/US/JP/DE/CN/ UK patent 2002)*

5. AA-Azatyrosine $\Rightarrow$ TW01 analogues *(US & 20 PCTs 2004)*

Inspiration 1: Ancestors' wisdom

Medicinal Chemist talks with the body.

---

drug + adjuvant drug + delivery system + vehicle
君　　臣　　佐　　使

Drug combination is functionally puzzle type rather than tweens.

Inspiration 2:

There is 君臣佐使.

Table 1. Modification of cyclosporin PK by TCMs.

	TCM-A	TCM-B	TCM-C
AUC	↑46 %	↑97 %	↑215 %
C_{max}	↑64 %	↑78 %	↑97 %

Table 2. Modification of Digoxin PK by TCMs.

	TCM-A	TCM-D	TCM-E
AUC	↑33 %	↑95 %	↑153 %
C_{max}	↑55 %	--	↑270 %
Porcine toxicity	Acute toxicity	Acute toxicity	Sudden death

~李珮端教授，台大生物醫學報導 2001~

Inspiration 3:

君臣佐使 could be beneficial.

IVAX's 圍魏救趙

p-Glycoprotein shipped taxol outbound.

⇒ Taxol is orally not absorbable.

⇒ Cyclosporin occupies p-glycoprotein

⇒ Taxol + Cyclosporin

⇒ Bingo!

⇒ However, IPR protection is limited.

⇒ NME is more attractive.

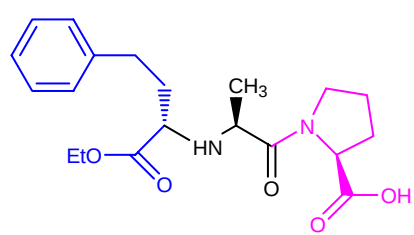
Inspiration 4: 911's 就地取材

ADME – Where to find delivery system (使)?

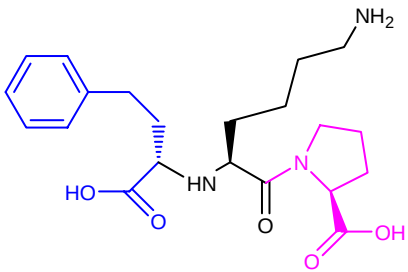
In search of seeing-eye dog (佐) to get on PepT1

Drug Metab Pharmacokinet. 2005, Annu Rev Physiol. 2004.

ACE inhibitors

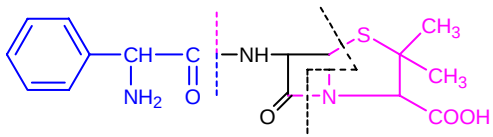


Enalapril

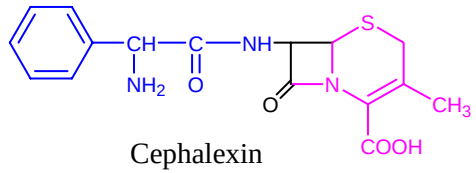


Lisinopril

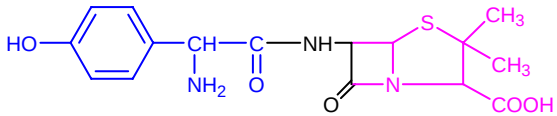
amino-β-lactams



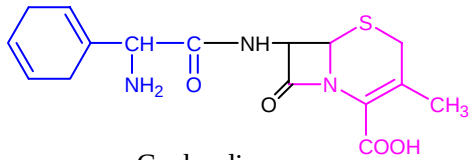
Ampicillin



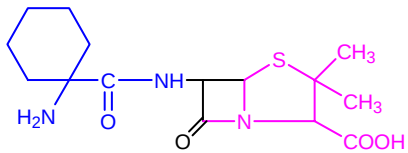
Cephalexin



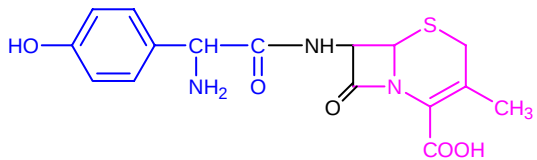
Amoxicillin



Cephradine



Cyclacillin

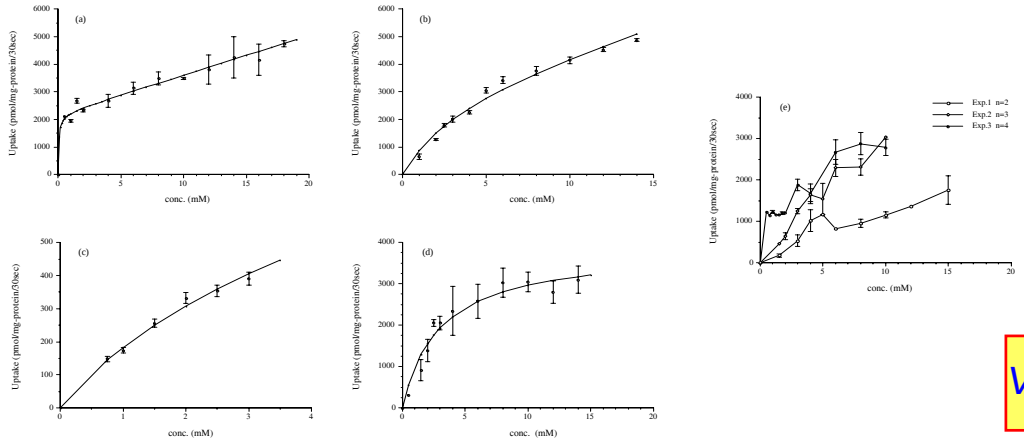


Cefadroxil

Fig. 3. Passengers of PepT1 oligopeptide shuttle bus.

Theory building

PhG-Md



Md

$$V = \frac{Vmax.[S]}{Km+[S]} + Kd\,.\,[S]$$

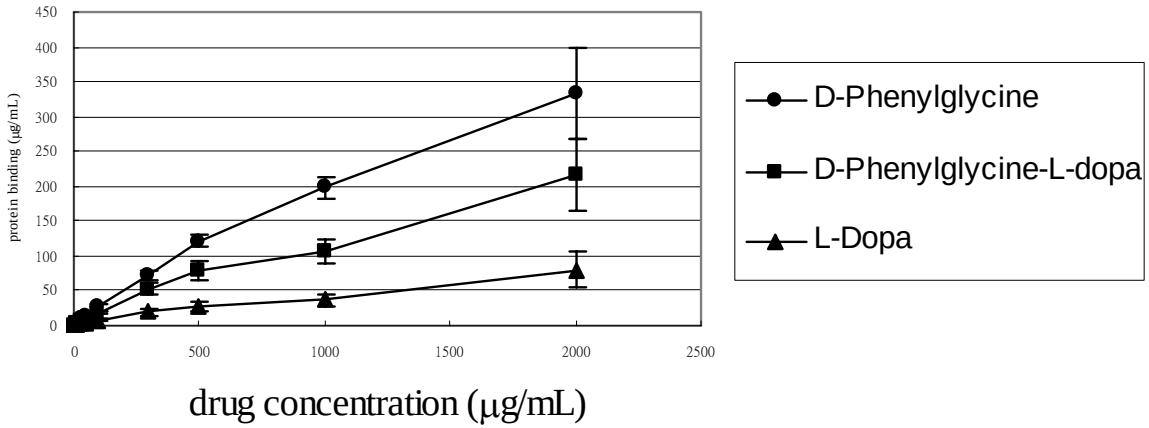
Fig. 4. BBMV uptake of di- and tripeptides of α-methyldopa.

Table 3. Michaelis-Menton parameters of di- & tripeptides.

Compound	n	K_m (mM)	V_{max}	V_{max}/K_m	K_d
PhG-Md	6	0.06 ± 0.13	2.18 ± 0.28	36.38	0.14 ± 0.02
HOPhG-Md	6	3.52 ± 0.60	3.12 ±0.19	0.89	0.19 ± 0.01
PhG-ALa-Md	6	2.24 ± 0.31	0.41± 0.08	0.18	0.58 ± 0.02
HOPhG-Pro-Md	6	2.97 ± 0.65	3.84 ± 0.28	1.29	0
PhG-Md (Amidon)		--	--	1.9	--

Inspiration 5:

ADME – What seeing-eye-dog (佐) can lead drugs to body bank for storage?



Compounds	binding on albumin (%)	First binding site	
		Ka_1 (L/mol)	n_1 (sites/L)
D-Phenylglycine	25.97 ±4.21	0.55	1.32
D-Phenylglycine-L-dopa	18.22 ±1.34	1.12	3.41
L-Dopa	7.62 ±1.44	0.29	0.59

Table 4. D-Phenylglycine, when chemically bound to L-dopa, increased its degree of albumin-binding.

YS Chaung, thesis, 2003

Inspiration 6:

ADME -- Is there ATM Cashing Box?

***P* might help *D* to retain longer in circulation.**

⇒ Metabolism functions as *ATM cash box.*

⇒ Small peptides are good approach.

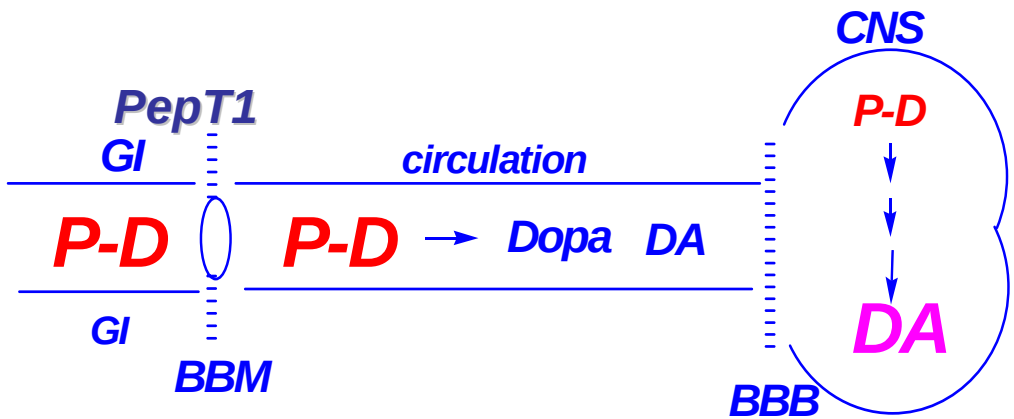


Fig. 5. The design of dopamine-releasing prodrugs.

D-Phenylglycine is a good seeing-eye dog (佐).

P-D: Oral BA ↑ 31 times. Vdss ↑ 30 times.

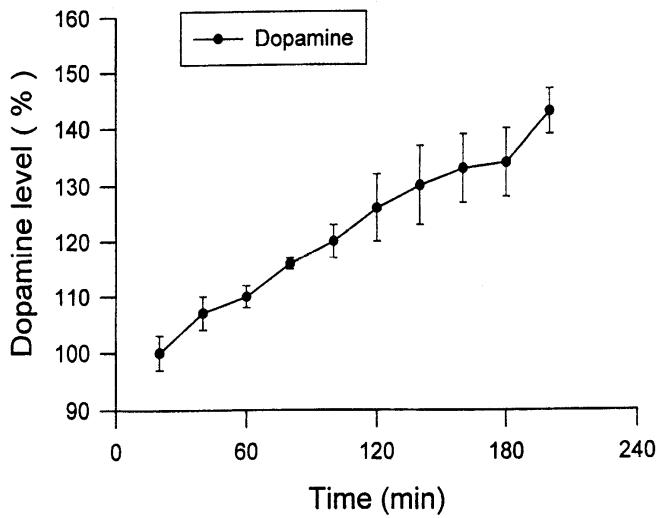
Table 5. L-dopa, bound to D-phenylglycine ends up with high oral BA and large Vdss.

	P-D	D-P	L-Dopa
AUC mg.min/ml	28.85±8.52	147.34±54.67	27.37±4.60
tmax(min)	38.3±17.7	23.0± 14.4	25.0±16.1
k (min -1)	0.005±0.001	0.0024±0.0005	0.004±0.001
t1/2(min)	142.5±23.7	300.3±56.6	184.80±46.20
Clp (L/kg/min)	0.29± 0.10	0.026±0.007	0.009±0.001
Vdss (L/kg)	35.7± 17.1	5.53± 2.17	1.22±0.36
BA (%)	27.58±4.56	16.0±3.74	0.87±0.24

Y. B. Fan master thesis, 1998.

P-D as intrinsic sustained dopamine-releasing prodrug.

(a) D-phenylglycine-L-dopa



(b) L-dopa

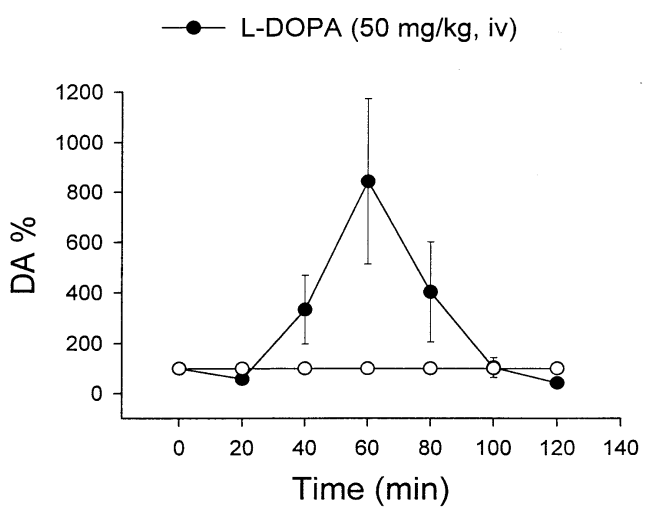


Fig.6. Striatal dopamine after i. v. injection of test drugs.

~Wang, H. P. et al US patent 1997~

Unfortunately:

No PepT1 shuttle bus on cell membrane for HPW98-1.

How to increase the $t_{1/2}$ of HPW98-1?

1. **Formulation ⇒ very little improvement for antitumor activity.**
2. **Dipeptide D-Phenylglycine-Azatyrosine showed no activity.**
3. **Chemical+physical modification ⇒ TW01 analogues**

Table 6. PK of HPW98-1.

N = 6	Mean ± SD
AUC (ug*min/mL)	179.59 ± 55.70
AUMC (ug*min2/mL)	4675.99 ± 1688.41
MRT (min)	26.52 ± 7.74
K (min-1)	0.04 ± 0.01
CLp (L/kg/min)	0.08 ± 0.02
Vdss (L/kg)	2.04 ± 1.00
t1/2 (min)	18.38 ± 5.37

Modified dipeptides led to TW01 analogues

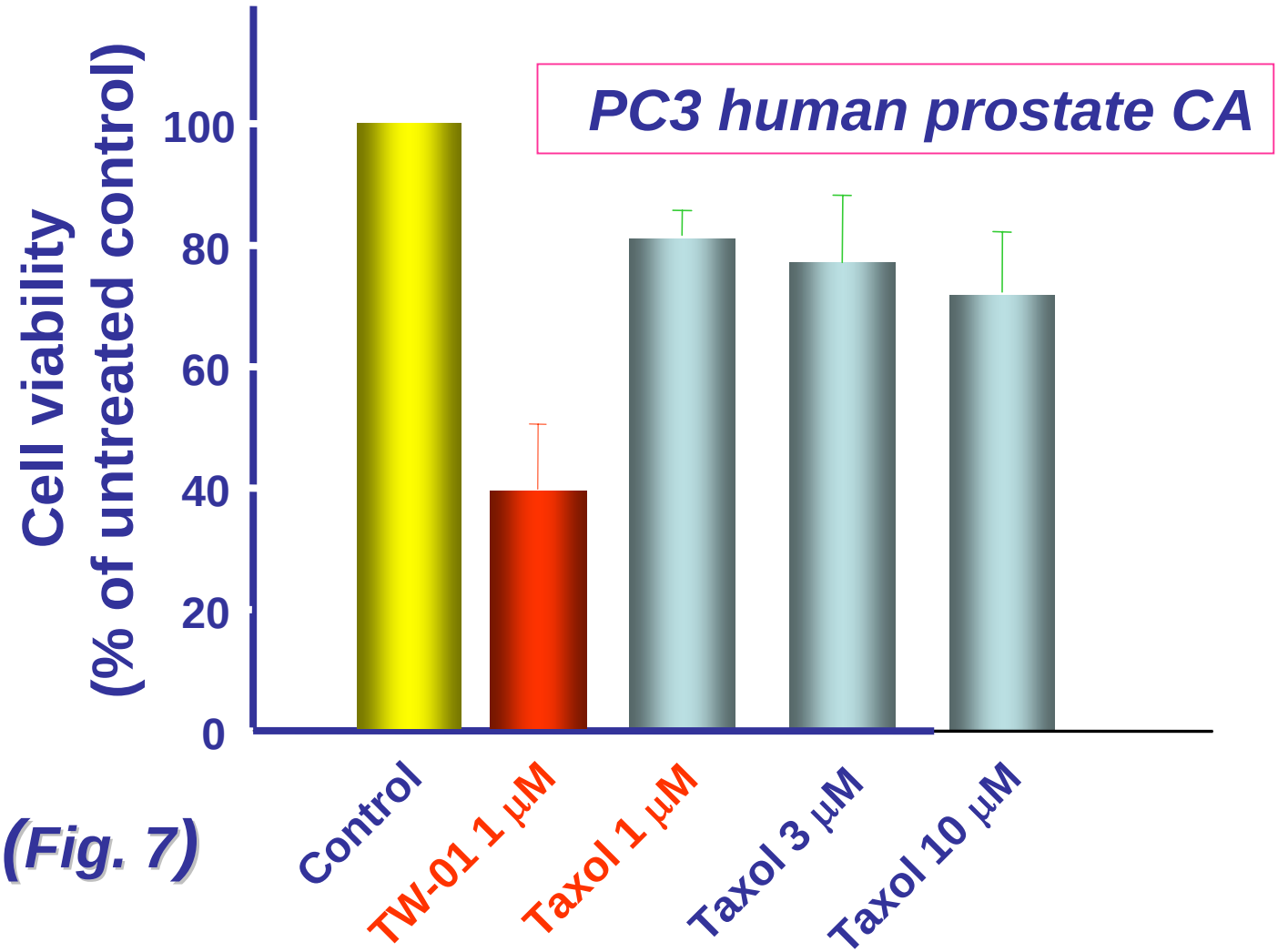
Seven out of eleven most active analogues

Table 7. In vitro activities tested in NCI and NTUMC.

Compound	Colorectal HT-29	Colorectal HCT116	Prostate PC-3	Hepatoma Hep3B	Breast MDA MB-231	Anti-angiogenesis
HPW99-5	+++++	+++++	++++	+++++	+++++	++++
HPW99-6	+	+++	+++	--	++++	++++
HPW99-12	+++	+++	++++	+++	+++++	+++
HPW99-13	+++++	+++++	++++	+++++	+++++	++++
HPW99-17	++++	++++	+++	++++	+++++	++++
HPW99-18	+++++	+++++	++++	+++++	+++++	++++
HPW99-20	+++++	+++	+++	+++	+++++	+++

+++++: $<10^{-7}$ M ++++: $<10^{-6}$ M +++: $<10^{-5}$ M +>10^{-5} M

TW01 is more active than taxol.



(Fig. 7)

TW01 is more active than taxol.

Table 9. *Comparison of TW01 and taxol on drug resistance human CA cell lines.*

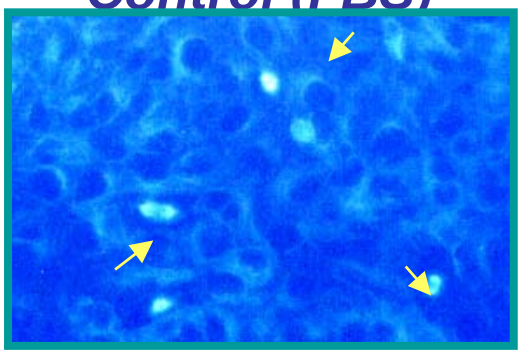
Cell line	IC_{50} (M)		Potency ratio
	TW01	Taxol	TW01/Taxol
Colorectal cancer			
HCT-15	4.0×10^{-8}	2.1×10^{-7}	5.2
Renal cancer			
ACHN	9.7×10^{-8}	1.5×10^{-6}	15.4
CAKI-1	6.3×10^{-8}	2.0×10^{-7}	3.1
TK-10	3.8×10^{-8}	6.9×10^{-8}	1.8
UO-31	5.1×10^{-8}	1.0×10^{-6}	19.6
Breast cancer			
MCF7/ADR-RE	2.6×10^{-8}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

Mechanism different from Taxol

Disruption of Mitotic Spindle Formation (Fig. 8)

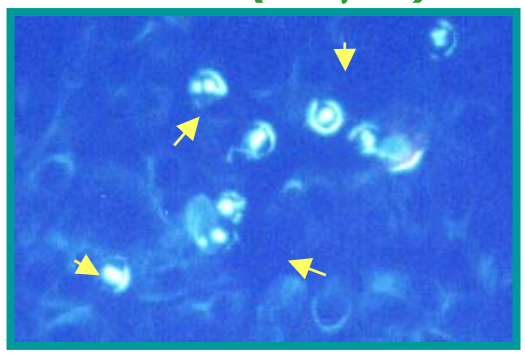
PC-3 prostate CA

Control (FBS)



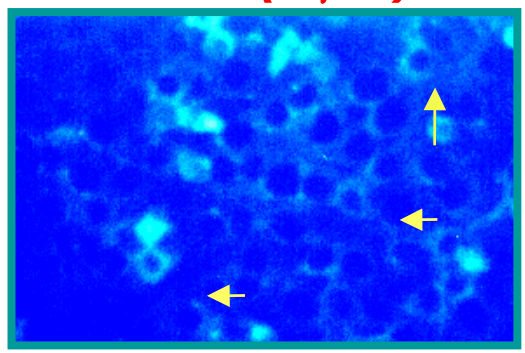
mitotic reaction

Taxol (10 μM)



Stabilize polymerization

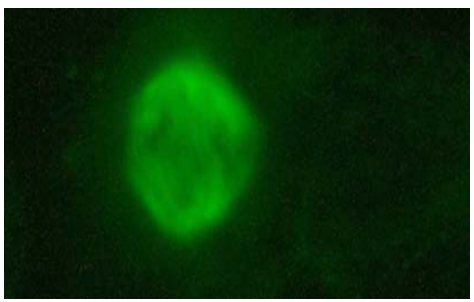
TW-01 (1 μM)



depolymerization

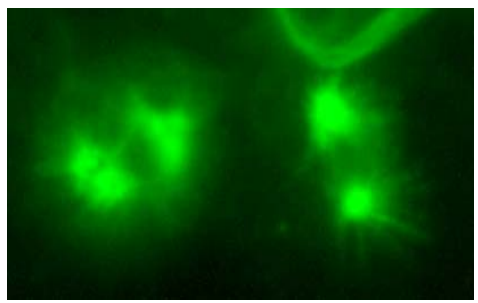
HA22T hepatoma

Control



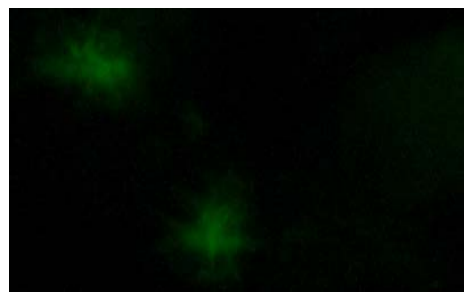
mitotic reaction

Taxol 1uM



Stabilize polymerization

TW-01 1uM



depolymerization

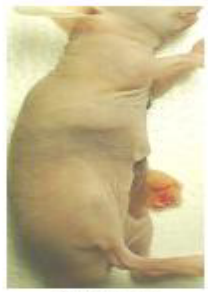
Comparison between HPW98-1 & TW01

HP Wang etal, US patent & PCT 2002.

HPW98-1 ⇒ HPW99-5 ⇒ in vitro potency ⇑ 100 times

TW-01
100 mg/kg,po

TW-01
30 mg/kg,po



TW-01
10 mg/kg,po

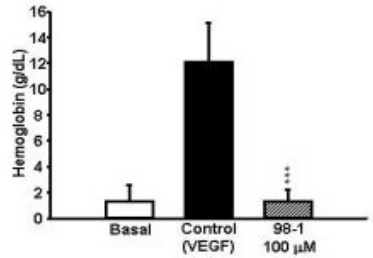


Fig 9. Hemoglobin count after treatment for 14 days.

Table 8

N=6	HPW98-1, iv	HPW99-5, iv
AUC (ug*min/mL)	179.59±55.70	64.8±33.0
K (hr^{-1})	2.4±0.6	0.36±0.14
CL_p (L/kg/hr)	4.8±.12	0.77±0.37
Vdss (L/kg)	2.04±1.00	13.8± 0.71
t1/2 (hr)	0.31±0.09	2.34±1.31

Wide safety margin of TW01

Activity vs toxicity of TW01: 10^{-7}~10^{-8} M vs 10^{-4}~10^{-5} M

Toxicity of control vs TW01: 10^{-7}~10^{-8} M vs 10^{-4}~10^{-5} M

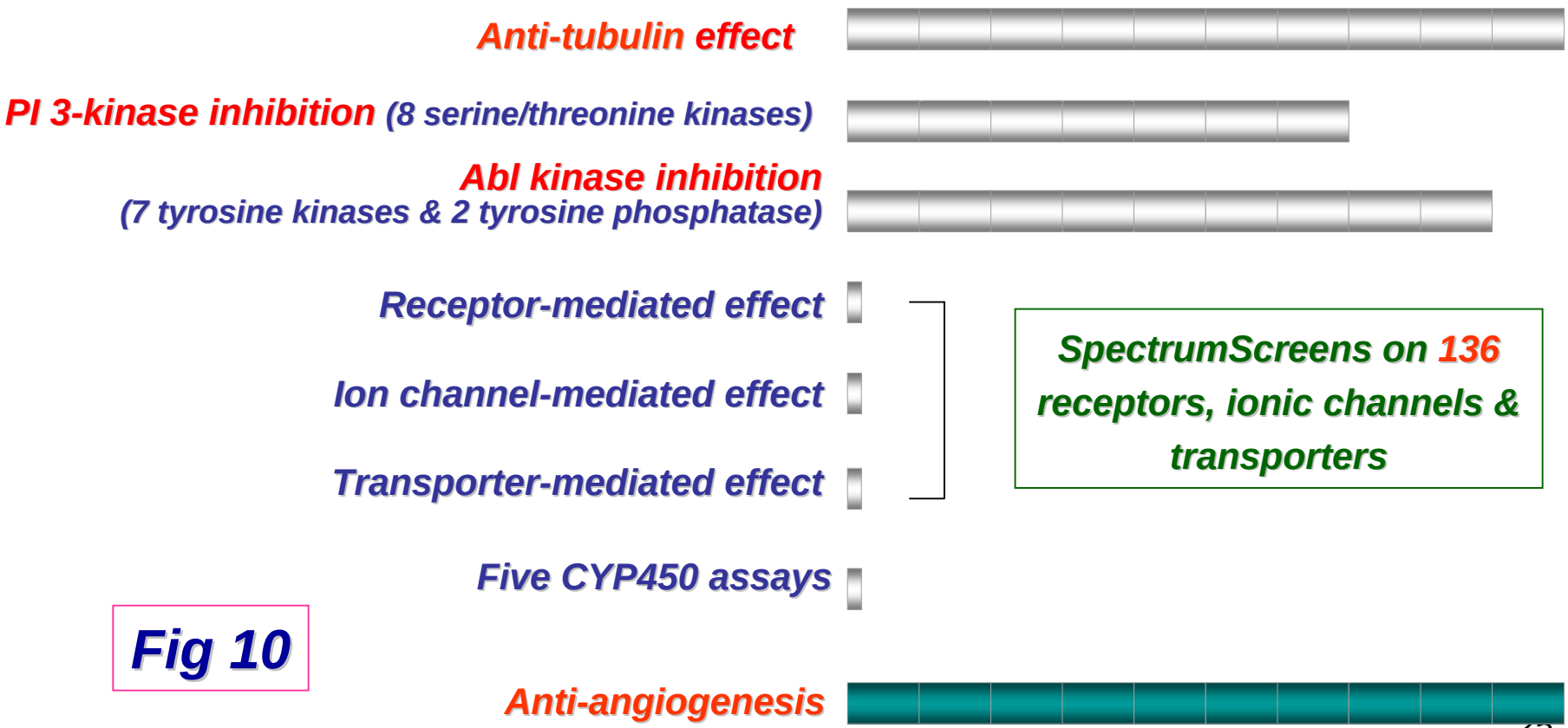


Fig 10

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

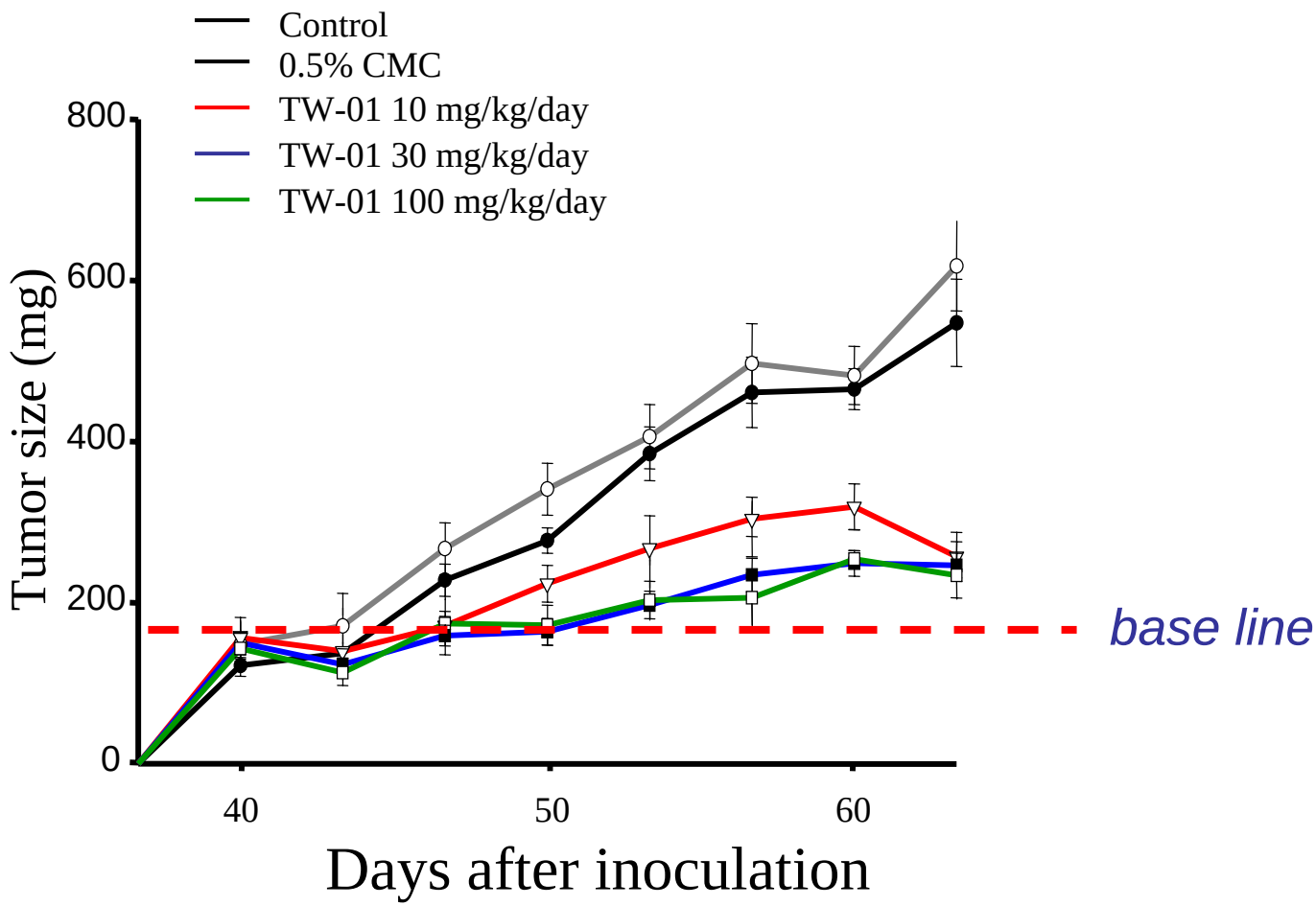


Fig 11

Survival days of mice bearing HA22T human hepatoma.

Table 11

	days (n=10)
Control	***70***
Doxorubicin	***88***
TW01 (3 mg/kg)	***74***
TW01 (10 mg/kg)	***78***
TW01 (30 mg/kg)	***>220***

T/C=130 in mice bearing A549 human non-small cell lung cancer.

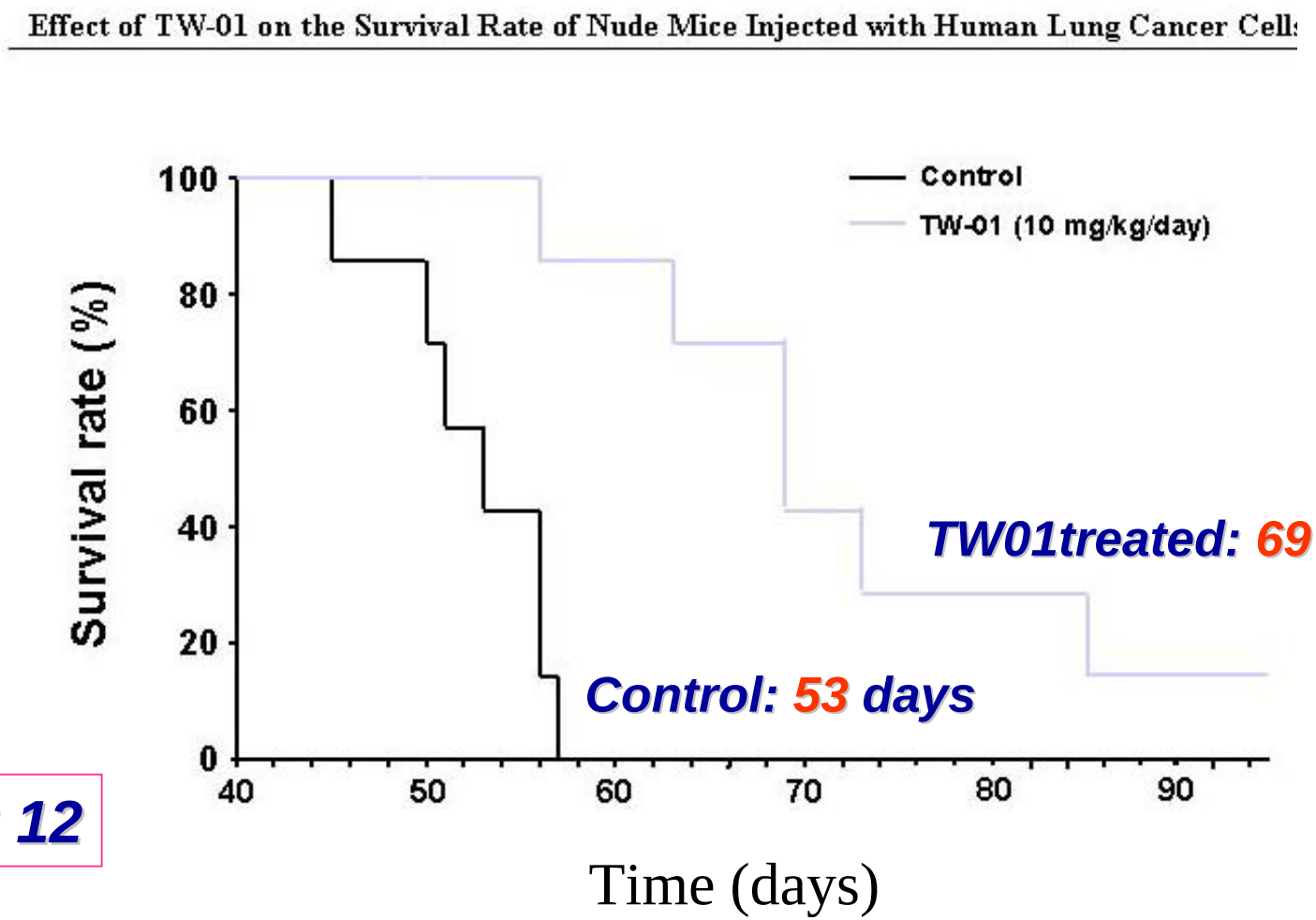


Fig 12

Moving forward

Table 12. PD/PK optimization of TW01

	In vitro IC_{50}	Oral BA %	Water solubility ug/mL
TW01	4.02×10^{-8} M	<1	23
BA-4	1.60×10^{-7} M	40.72±16.27	137± 6.26
BA-5	9.2×10^{-8} M	--	286.38±1.17
BA-6	3.7×10^{-7} M	--	197.59 ±0.63

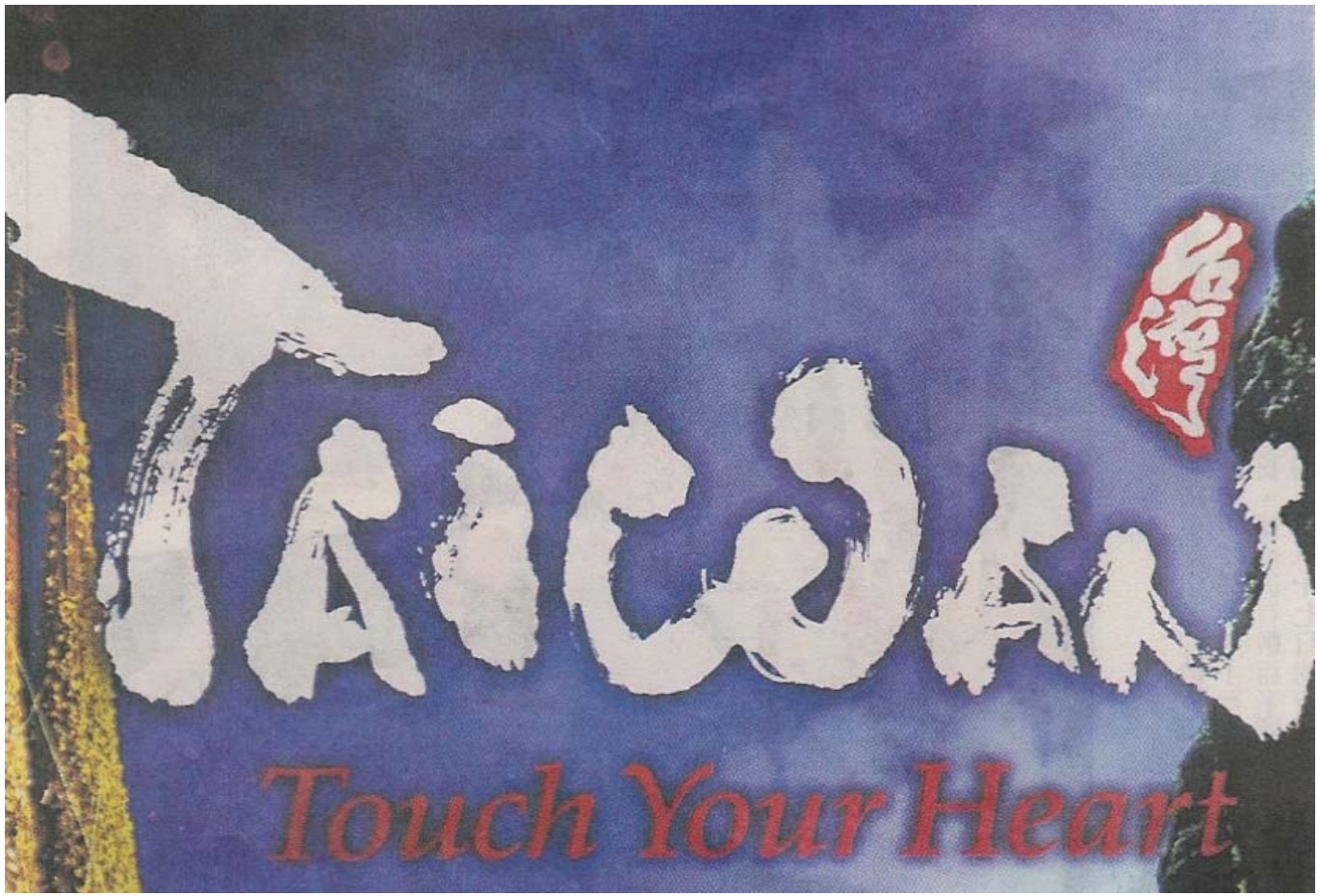
IPR Profile

Table 13

Granted	***Taiwan, USA, Singapore***
Published	***Brazil, China, Europe, Hungary, Indonesia, Korea***
Pending	***Australia, Canada, Czech, India, Japan, Malaysia, New Zealand, Russia, South Africa***

Conclusion

Cinderella's wonderland: Imagination



Talk with the body creates lots of fun.

Medicinal Chemist talks with the body.

- ***Away from the main stream is the chance for Cinderella.***
  - ***1. Not work on TCM per se.***
  - ***2. Adopt TCM theory (君臣佐使) in NCE design***
- ***ADME: Let body triggers drug action***
  - ***1. 911's model: 就地取材***
  - ***2. Intrinsically activated: 以子之矛攻子之盾***
  - ***3. Non-obviousness: 神不知鬼不覺***
  - ***4. Intrinsic sustained release***
  - ***5. Few additives, few xenobiotics,***
  - ***6. No polution, little burden to the body***

Face the reality as early as possible.

Play tricks is the way toward success.

人在公門 努力修行 練功三年 發功看我

三十六計新譯

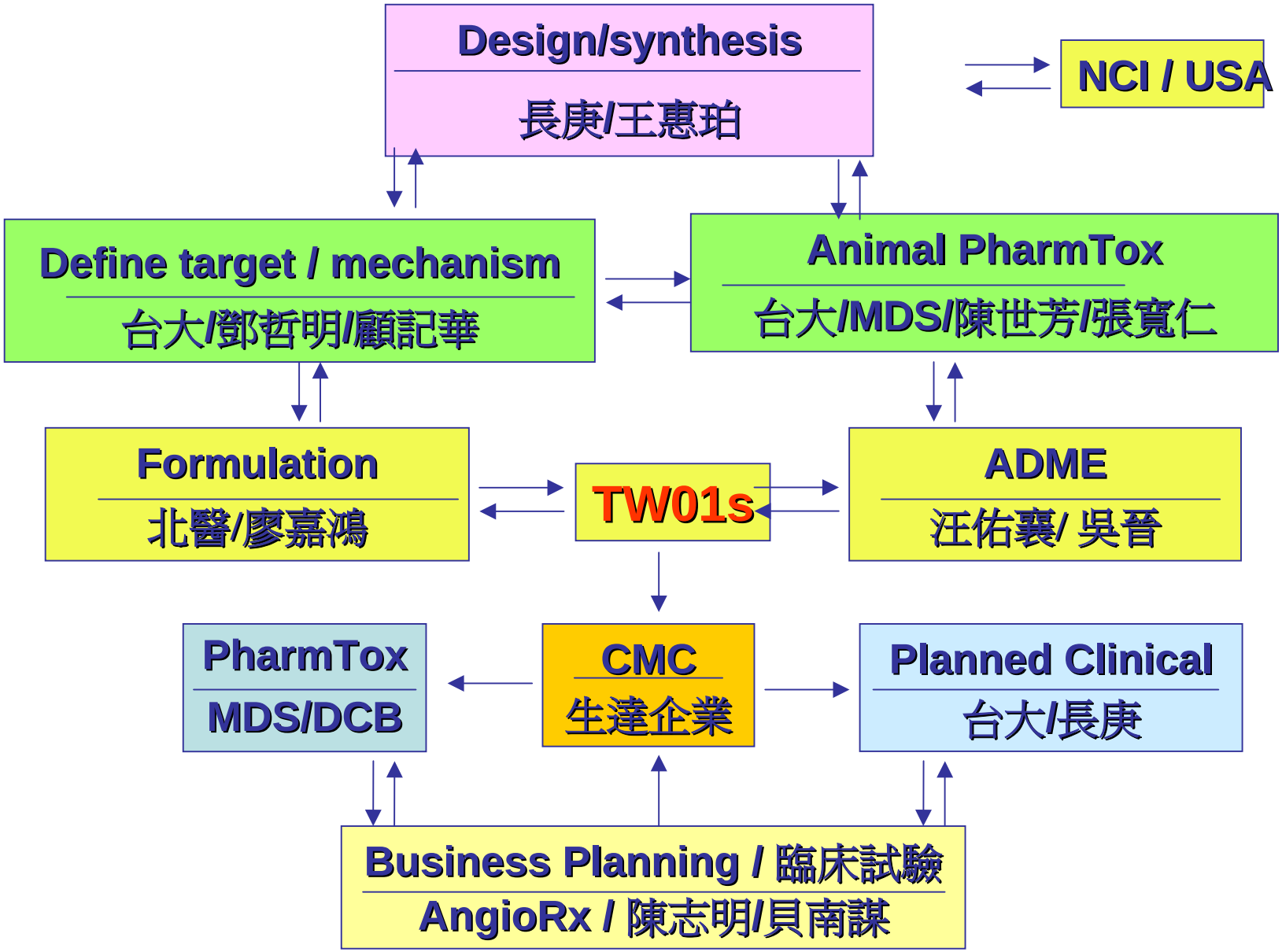
景福醫訊 1995, 12, 78-81.

—中華文化與藥物設計—

王惠珀 駱文琳

"西學中用" 主導中國歷史數百年，到屆臨二十一世紀的今天，東風西漸，以全然不同的風采吹向西方。可以見到的，例如氣功的風靡，草藥(Chinese herbal medicine)的流行，1/3 美國人有使用草藥的經驗(Eisenberg, New Eng. J. Med. 1993)，草藥正以每年15%的成長率帶動美國健康食品市場(Brevoort, Rapid utilization of natual products, 1994)。兩岸科學及衛生界熱衷提倡科學中藥以及中藥科學化研究。國科會甚至有生物能的研究。老祖宗留下的文化精髓中到底還有什麼錦囊妙計，讓我們這群學院派西tetracycline等均是。大多數發展出來的藥物，其作用方式屬於直搗黃龍，達到反客爲主(三十六計之三十)的陣勢。唯反客爲主頗傷腦筋，需以多取勝(因係reversible binding)，否則會像台灣這五十年的時移事易一般，經過兩番的反客爲主(又稱喧賓奪主三十六計之二十)，主流非主流角色互易，造成許多人適應不良，後遺症多多，終歸是笨方法。無怪乎聰明的老祖宗不把這種以多取勝的人海戰術歸於三十六計之列。有鑑於此，二十世紀初，藥物化學祖師Paul Erhlich提出了magic bullet的概念，畢竟子彈(irreversible

研發團隊


Design/synthesis
長庚/王惠珀
NCI / USA
Define target / mechanism
台大/鄧哲明/顧記華
Animal PharmTox
台大/MDS/陳世芳/張寬仁
Formulation
北醫/廖嘉鴻
TW01s
ADME
汪佑襄/ 吳晉
PharmTox
MDS/DCB
CMC
生達企業
Planned Clinical
台大/長庚
Business Planning / 臨床試驗
AngioRx / 陳志明/貝南謀

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Standard Co. Taiwan

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AngioRx Co.

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Dr. 許金玉　Ms. 陳惠婷

Dr. 胡幼圃　Mr. 莊雲翔

Dr. 吳銘芳　Mr. 謝緯賢

Moving forward:
Great fun or no fun?

Shel Silverstein~

THANK YOU