Univerzita Komenského v Bratislave
Prírodovedecká fakulta, Katedra organickej chémie

Prečo chemici púšťajú zlúčeniny žilou?

Bratislava, 7.11.2008

Andrej Boháč

Organická chémia

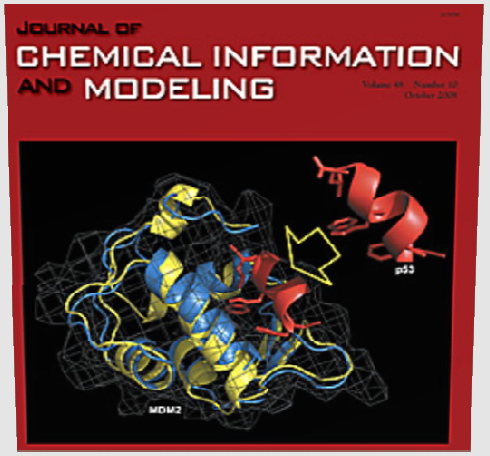
- náuka o spôsobe ako pripraviť nové zlúčeniny s novou kvalitou a vlastnosťami
- > 11 000 000 org zlúčenín (Beilstein)
  > 36 500 000 chem zlúčenín (SciFinder)
- chemical space > 10^{60} (možné a stabilné molekuly)

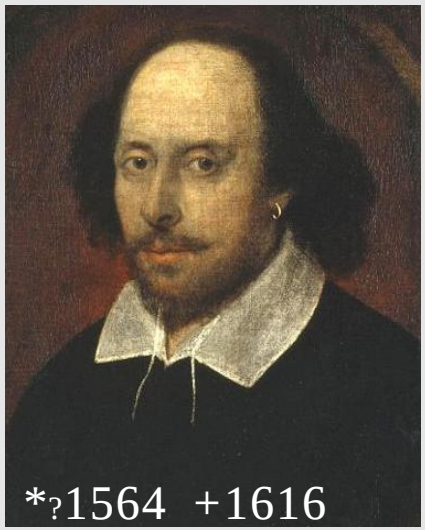
SI prefixes

1000^m	10^n	Prefix	Symbol	Since[1]	Short scale	Long scale	Decimal
1000^8	10^{24}	yotta-	Y	1991	Septillion	Quadrillion	1 000 000 000 000 000 000 000 000
1000^7	10^{21}	zetta-	Z	1991	Sextillion	Trilliard	1 000 000 000 000 000 000 000
1000^6	10^{18}	exa-	E	1975	Quintillion	Trillion	1 000 000 000 000 000 000
1000^5	10^{15}	peta-	P	1975	Quadrillion	Billiard	1 000 000 000 000 000
1000^4	10^{12}	tera-	T	1960	Trillion	Billion	1 000 000 000 000
1000^3	10^9	giga-	G	1960	Billion	Milliard	1 000 000 000
1000^2	10^6	mega-	M	1960	Million		1 000 000
1000^1	10^3	kilo-	k	1795	Thousand		1 000

?

- *Reymond J-L et al* ***J. Chem. Inf. Model. 47 2007 342.*** *11 Atoms of C, N, O, F: Assembly of 26.4 Million Structures (110.9 Million Stereoisomers)...".*




*?1564 +1616

To be or not to be?

Aké zlúčeniny pripravovať?

Trhová ekonomika a veda

- diktuje praktický výstup – interdisciplinárny charakter výskumu
- **materiálová chémia**
  (chémia – fyzika – matematika)
- **bioorganická chémia, medicínska chémia**
  (chémia – biológia – medicína)

Medicínska chémia

- vývoj a výskum liečiv

- Na čo majú nové zlúčeniny slúžiť?
  (medicína, molekulárna biológia)
- Aká je ich správna štruktúra?
  (medicínska chémia)
- Ako ich pripraviť? (organická syntéza)
- Sú to tie správne zlúčeniny?
  (biológia, medicína)
- Ako ich chrániť na trhu? (právo)

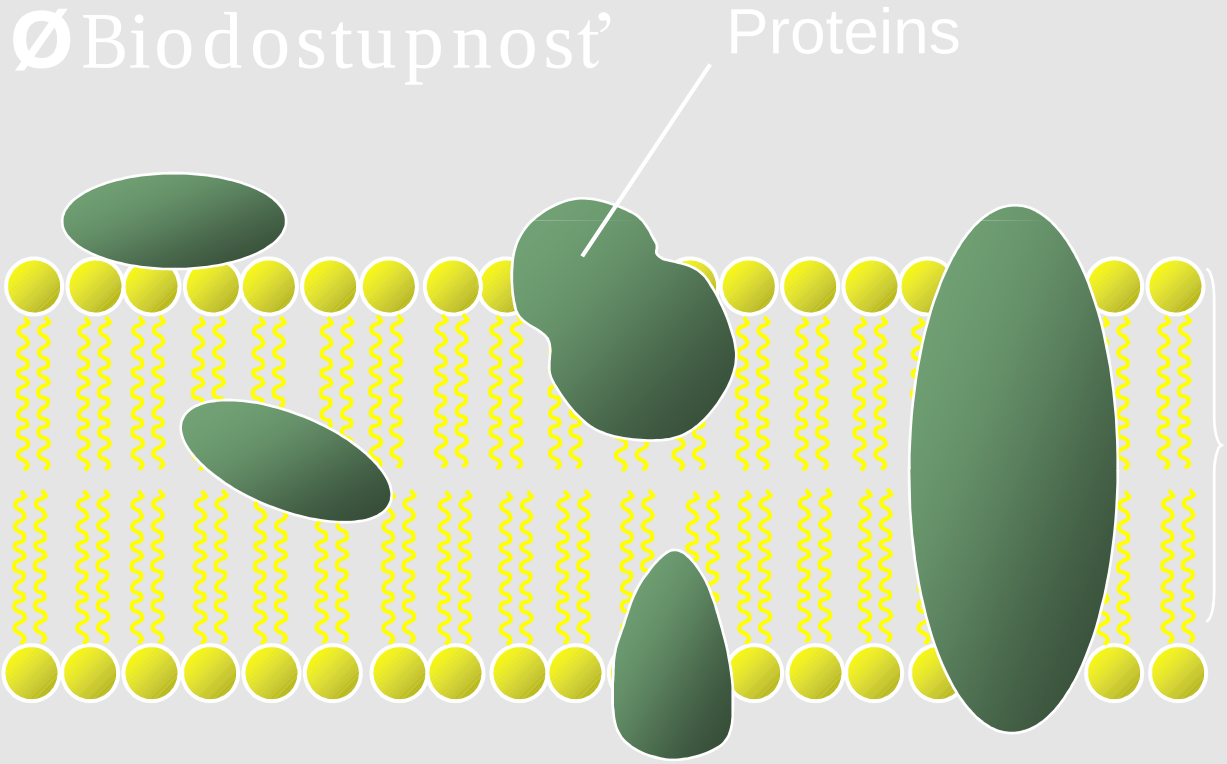
Vývoj nových liečiv

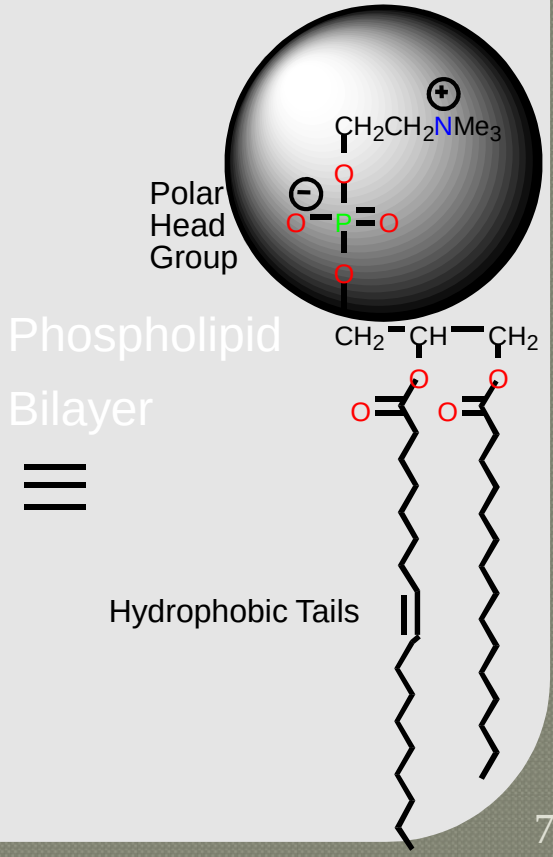
- vysoko interdisciplinárny a časovo náročný proces vyžadujúci ca 10 rokov a náklady ca 800 000 000 USD

- ročne len ca 26 nových liekov
  (2005: 26, 2004: 24, 2006: 26, 2002: 28)

Čo musí splniť liečivo?

- Aktivita (stereoelektronická kompatibilita)
- Nízka toxicita
- Biodostupnosť





Drug-like properties (druglikeness)

most drugs are relatively small and lipophilic molecules

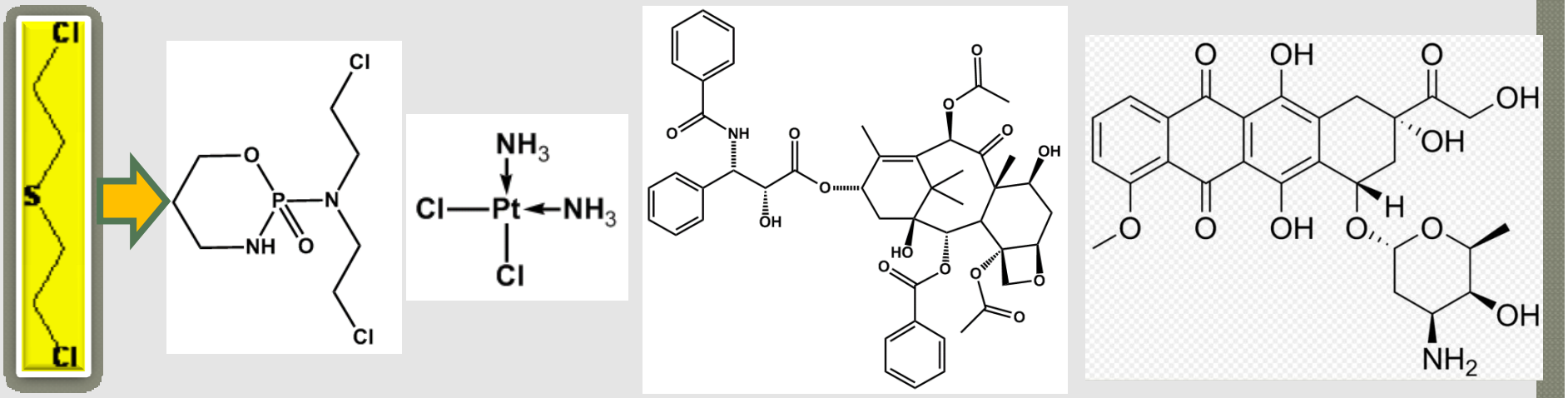
Lipinski´s rule of five: (1997)

FW < 500 g/mol
< 5 H-bound donor groups (HO-, HN-,HS-...)
< 10 H-bound acceptor groups (-O-,-N-,-C=O...)
log P < +5

1. výber ochorenia
2. poznanie molekulárneho mechanizmu
3. selekcia biologického cieľa (protein..., „*target*")
4. predklinické testy (*in vitro, in vivo*)
5. design,nosná štruktúra (In Silico design, Click chemistry, drug like, toxicology, ADME)
6. syntéza a optimalizácia aktivity a selektivity
7. klinické testy (PhI-III, IV)
8. priemyselná optimalizácia výroby
9. patentovanie
10. administratívne schválenie (FDA, ŠUKL)
11. klinická prax

Liečba onkologických ochorení

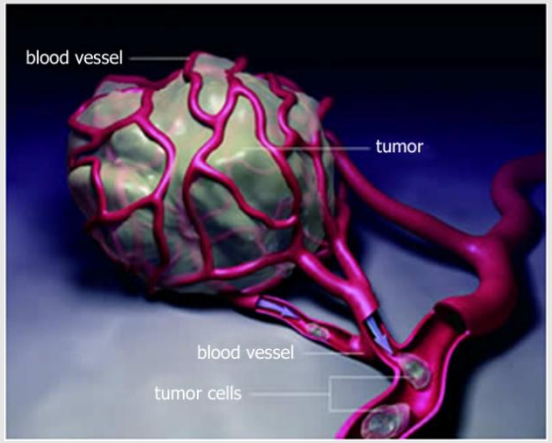
- Chirurgická
- Rádioterapia
- Chemoterapia (cyklofosfamid, cis-platina, taxol, doxorubicín…vincristín…)



- Antiangiogénna liečba (2004/2005/2006) (bevacizumab, sorafenib tozylát, sunitinib malát)

ANGIOGENÉZA

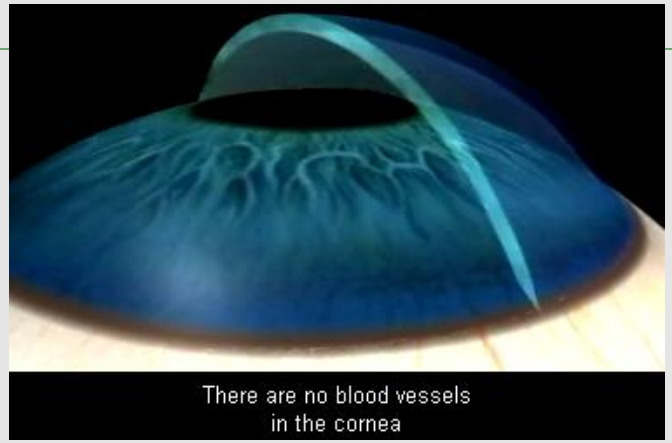
- novotvorba ciev z existujúcich ciev
- 1971 J. Folkman hypotéza ⇨ tumory sú angiogénne závislé

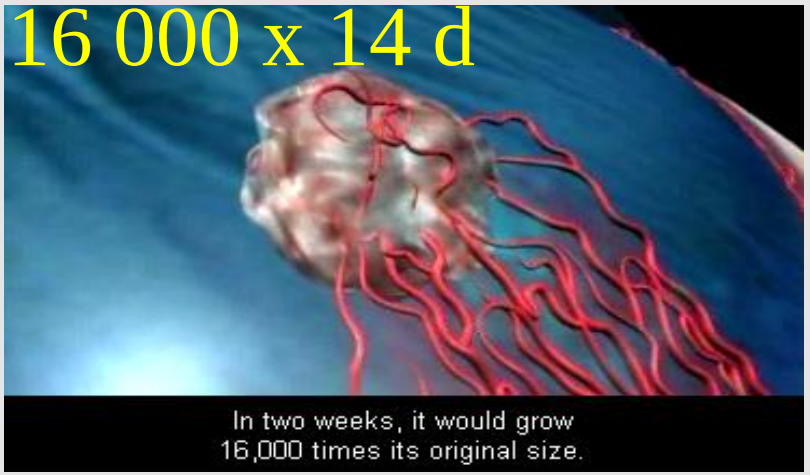


Dr. Judah Folkman 1933 – 2008

…..“Angiogenesis research will probably change the face of medicine in the next decades……...”

Experimentálny dôkaz

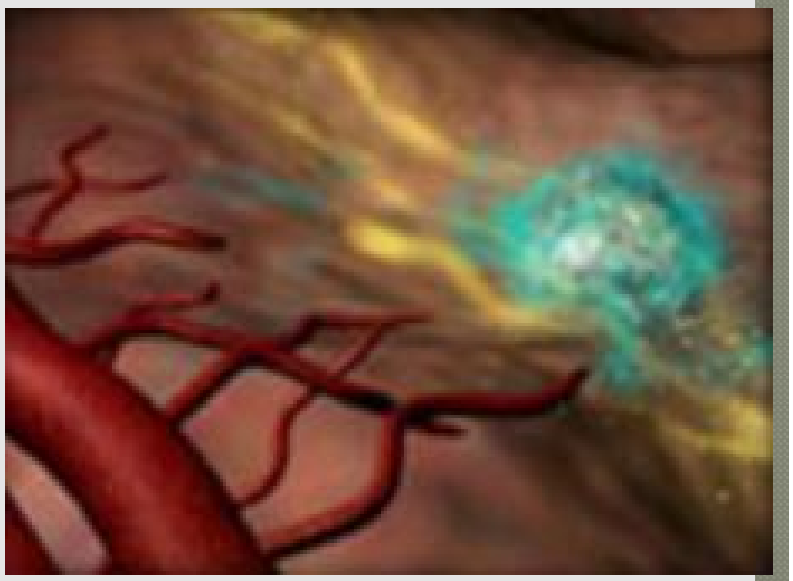


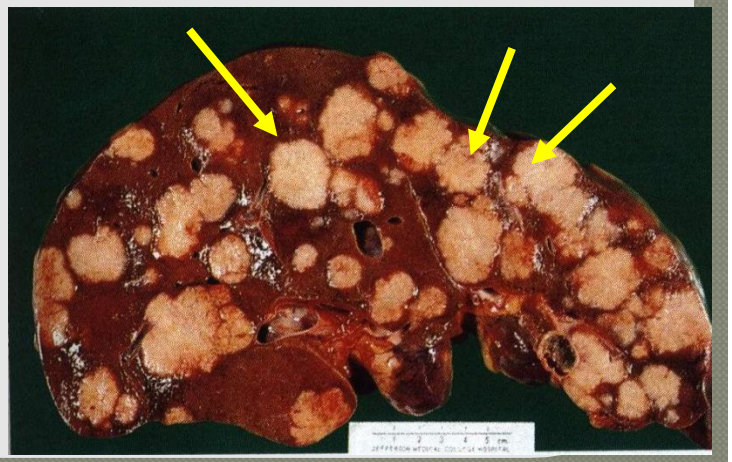


tumor produkuje promotory angiogenézy

Evolúcia tumoru a angiogenéza

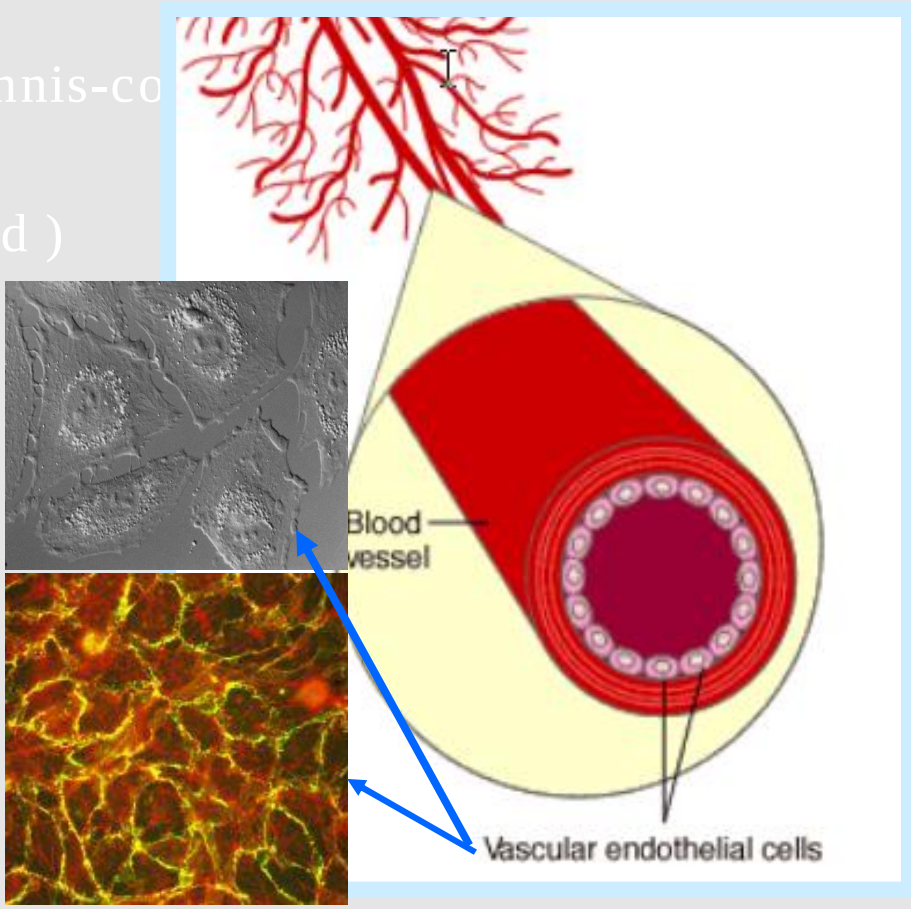
- prekanceróza
- tumor „*in situ*“ 0.2-2mm (10^6 imortalizovaných buniek), rovnováha (proliferácia / nekróza, apoptóza)
- angiogény fenotyp („angiogenic switch“)
- neovaskularizácia tumoru
- progres, metastázy



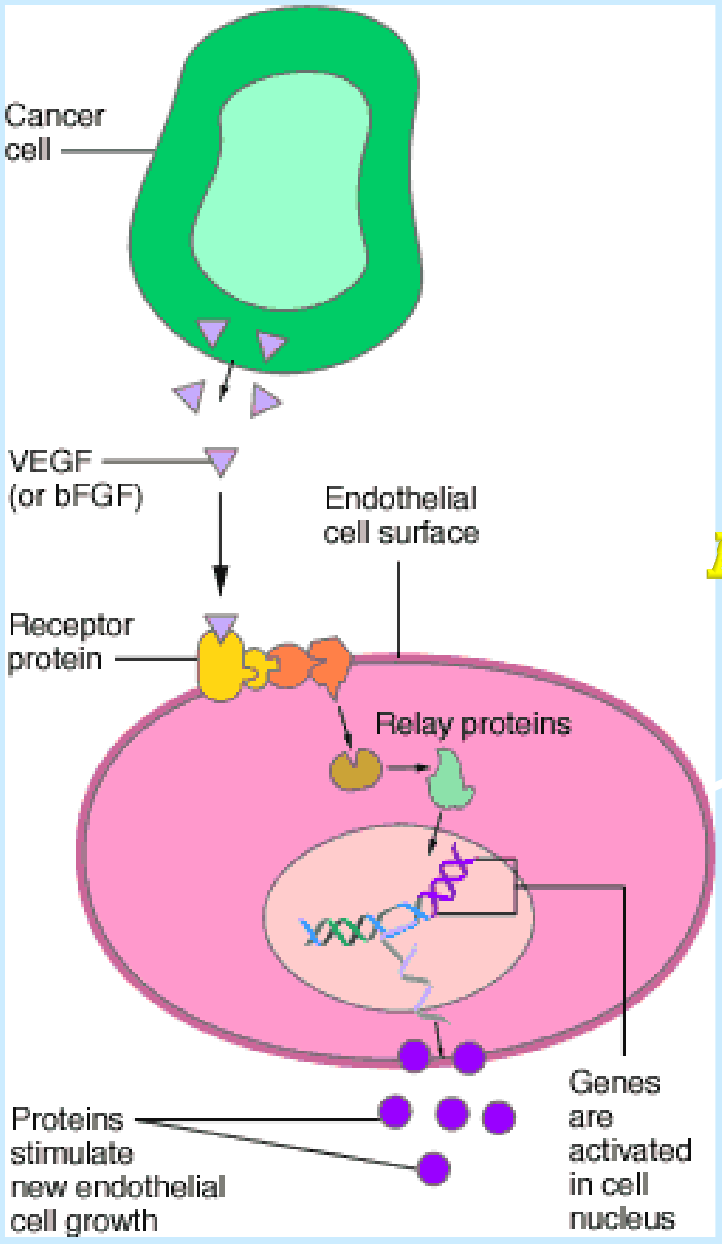


Blood-Vessels

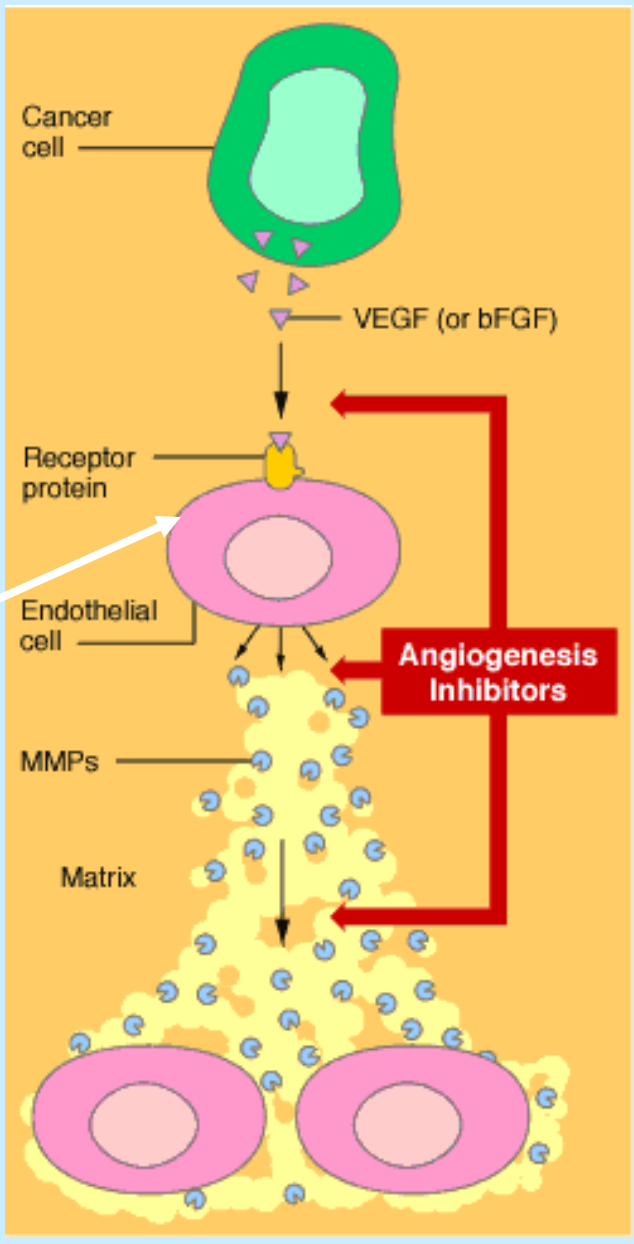
- **Endothelial Cells** (EC)
  - endothelium ca 1 000 m^2 ≈ tennis-co
  - flat cells, tile shape
  - divide a´2-3 years (tumor a´2 d)
- **Basement Membrane**
- **Smooth Muscle Cells**
- **Pericytes**



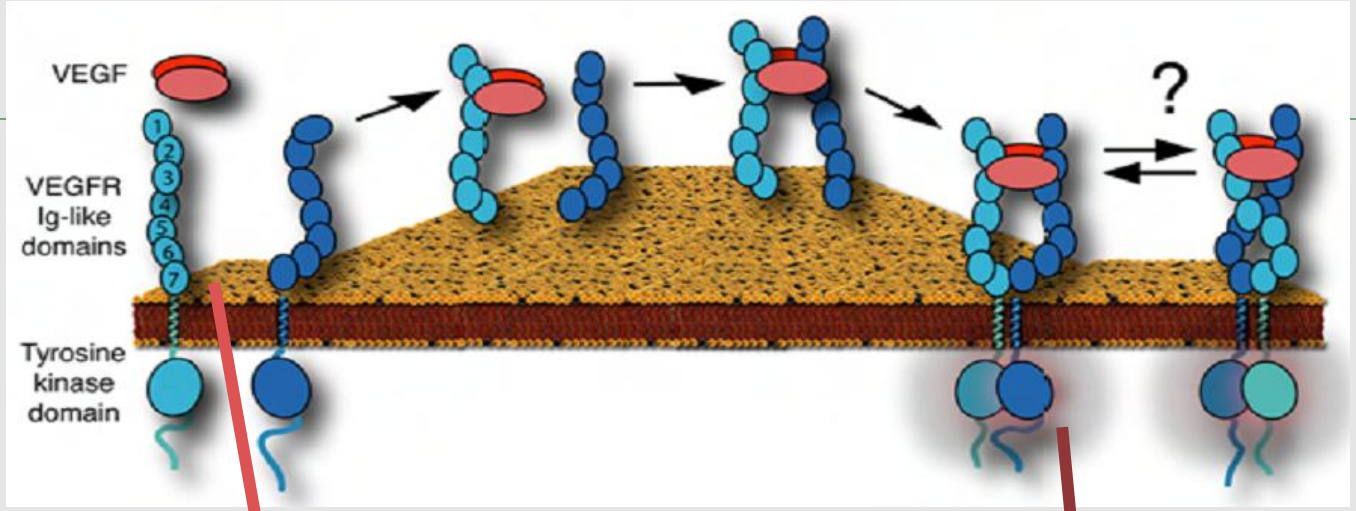
Selekcia biologického cieľa



Aktivácia EC



VEGFR-2 (KDR, Flk-1)



KDR (VEGFR-2, Flk-1) 1337AA (No. 20-1356)

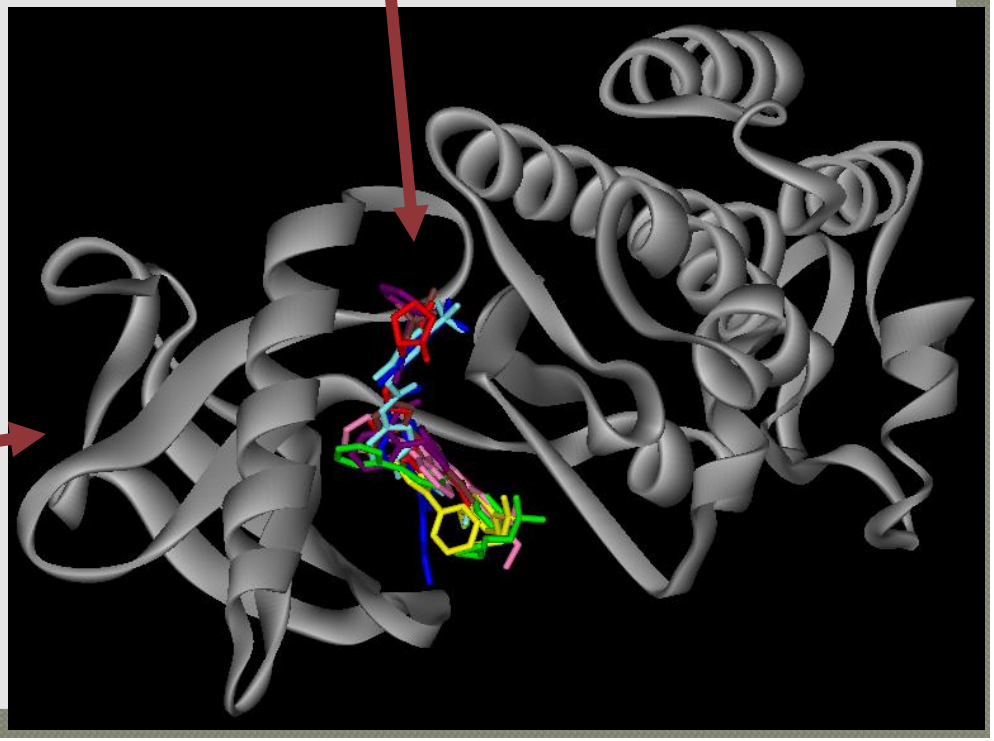
151 kDa
monomeric

VEGF binding part (Ig-like C2 type 1-7)
745 AA (No. 20-764)

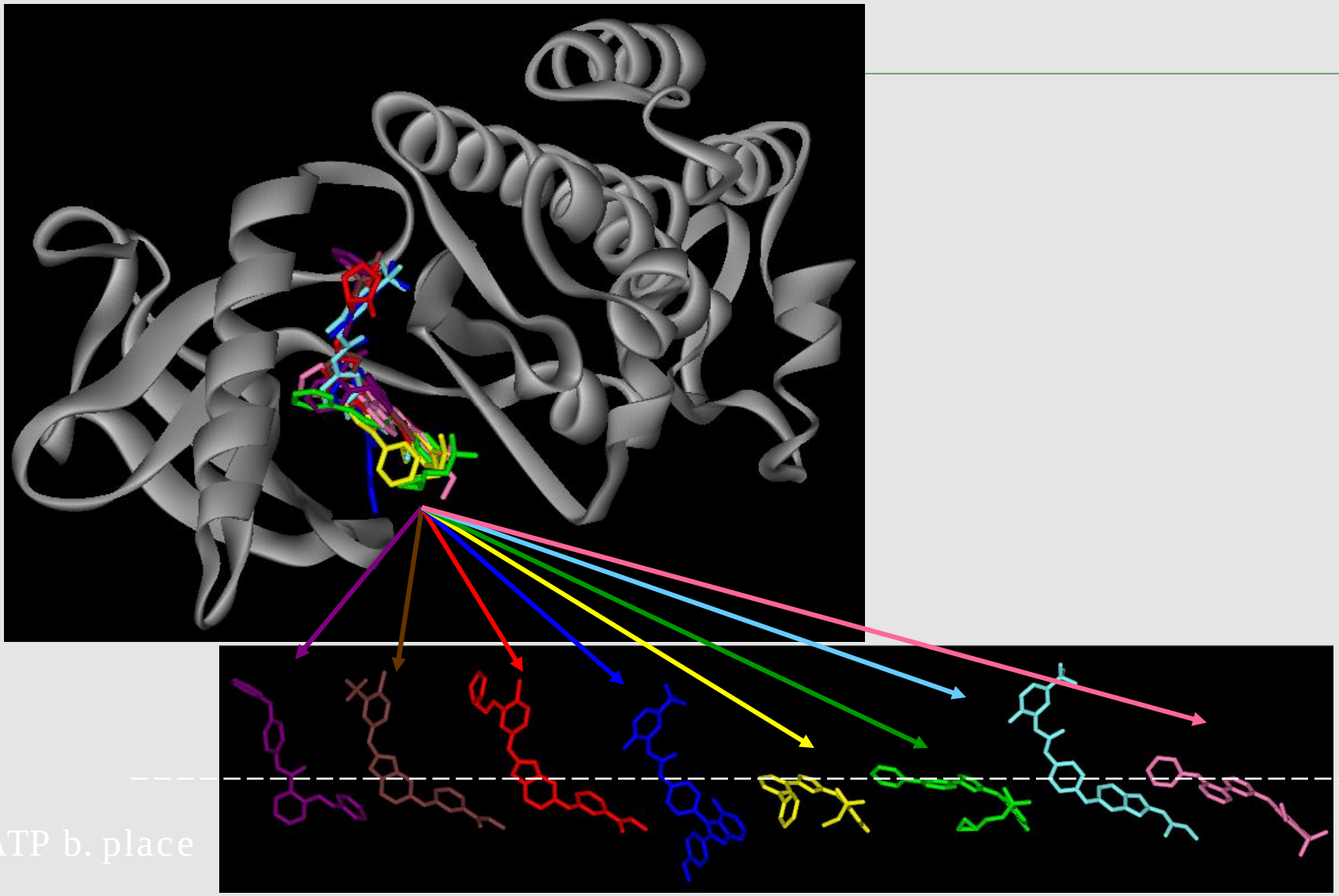
25AA (No. 765-789)

567 AA (No. 790-1356)
TK - activity

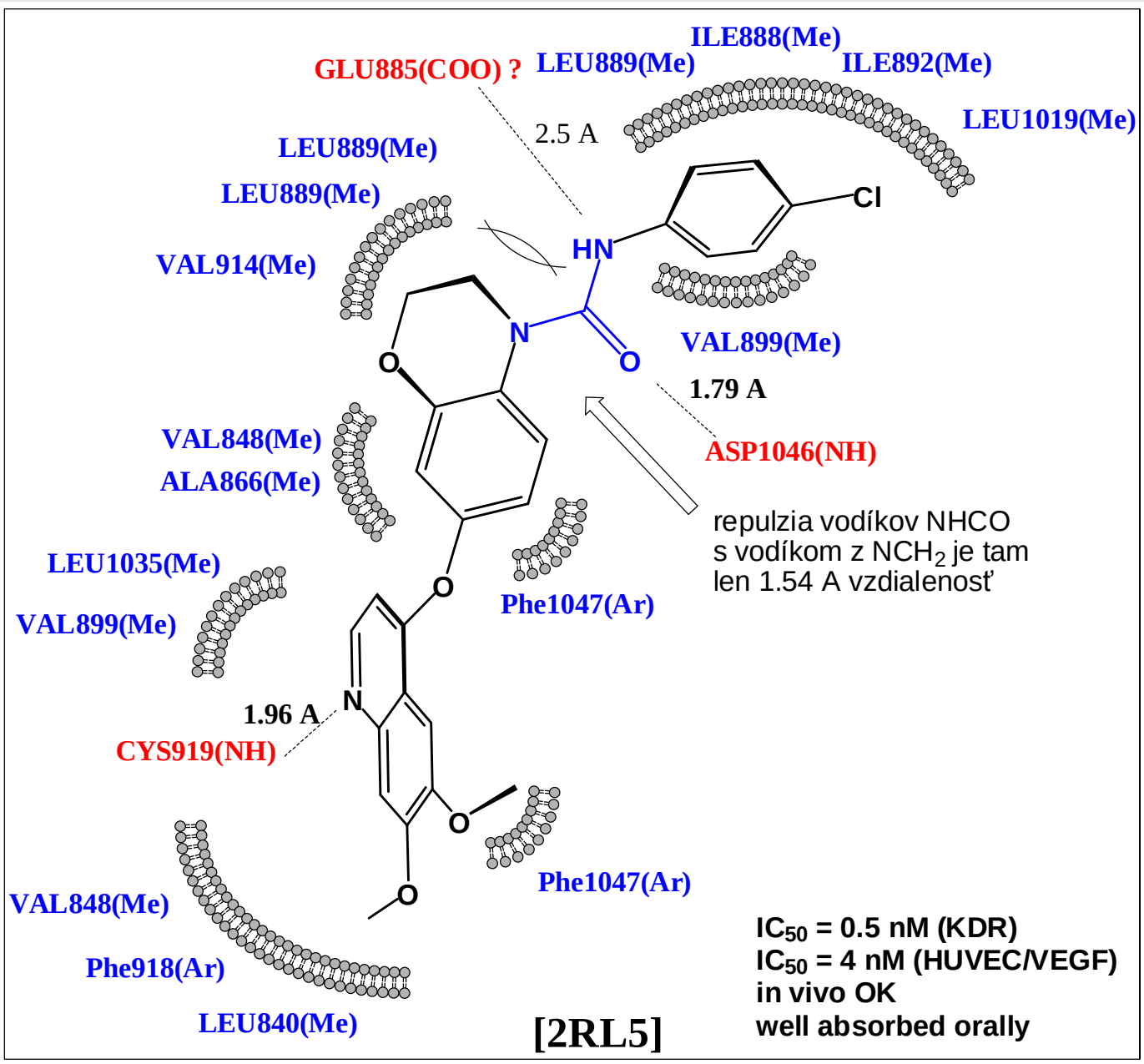
329 AA is TK (No. 834-1162)



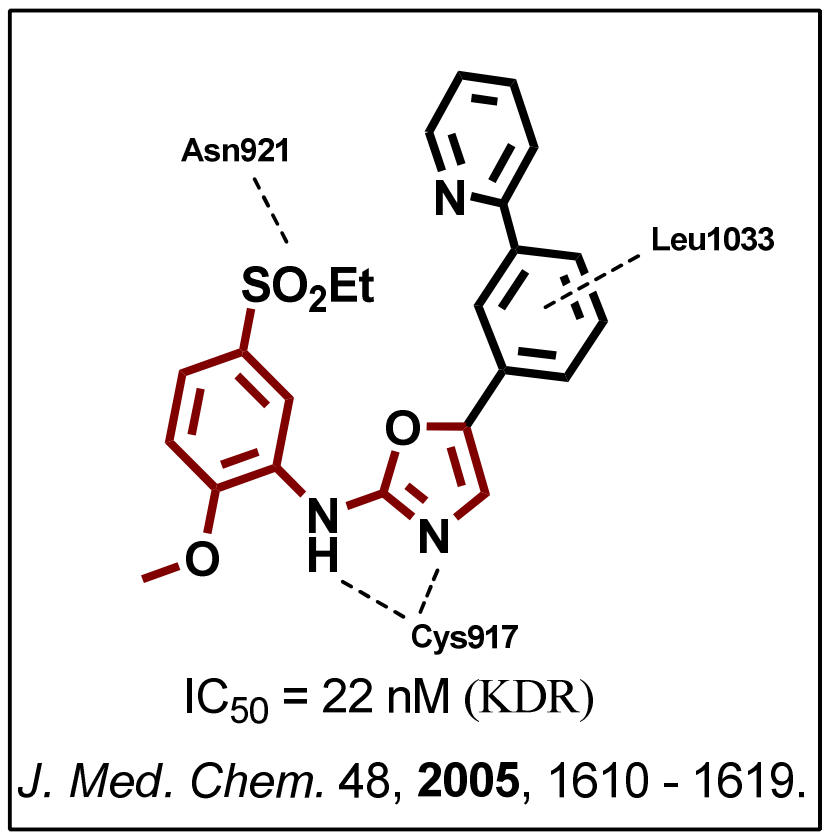
X-ray analysis



ATP b. place

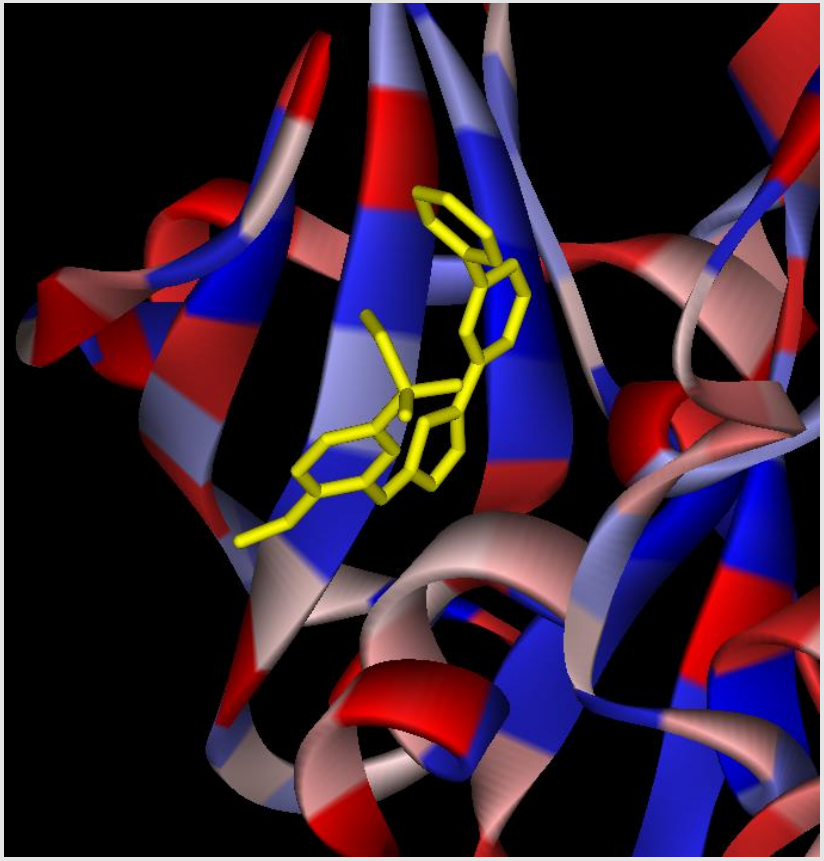

ILE888(Me)
GLU885(COO) ?
LEU889(Me)
ILE892(Me)
LEU1019(Me)
2.5 A
LEU889(Me)
LEU889(Me)
VAL914(Me)
Cl
HN
N
O
O
VAL899(Me)
1.79 A
ASP1046(NH)
VAL848(Me)
ALA866(Me)
repulzia vodíkov NHCO
s vodíkom z NCH2 je tam
len 1.54 A vzdialenosť
LEU1035(Me)
VAL899(Me)
O
Phe1047(Ar)
N
1.96 A
CYS919(NH)
O
O
Phe1047(Ar)
VAL848(Me)
Phe918(Ar)
LEU840(Me)
[2RL5]
IC50 = 0.5 nM (KDR)
IC50 = 4 nM (HUVEC/VEGF)
in vivo OK
well absorbed orally

KDR inhibítor (1 mg / 100 L)



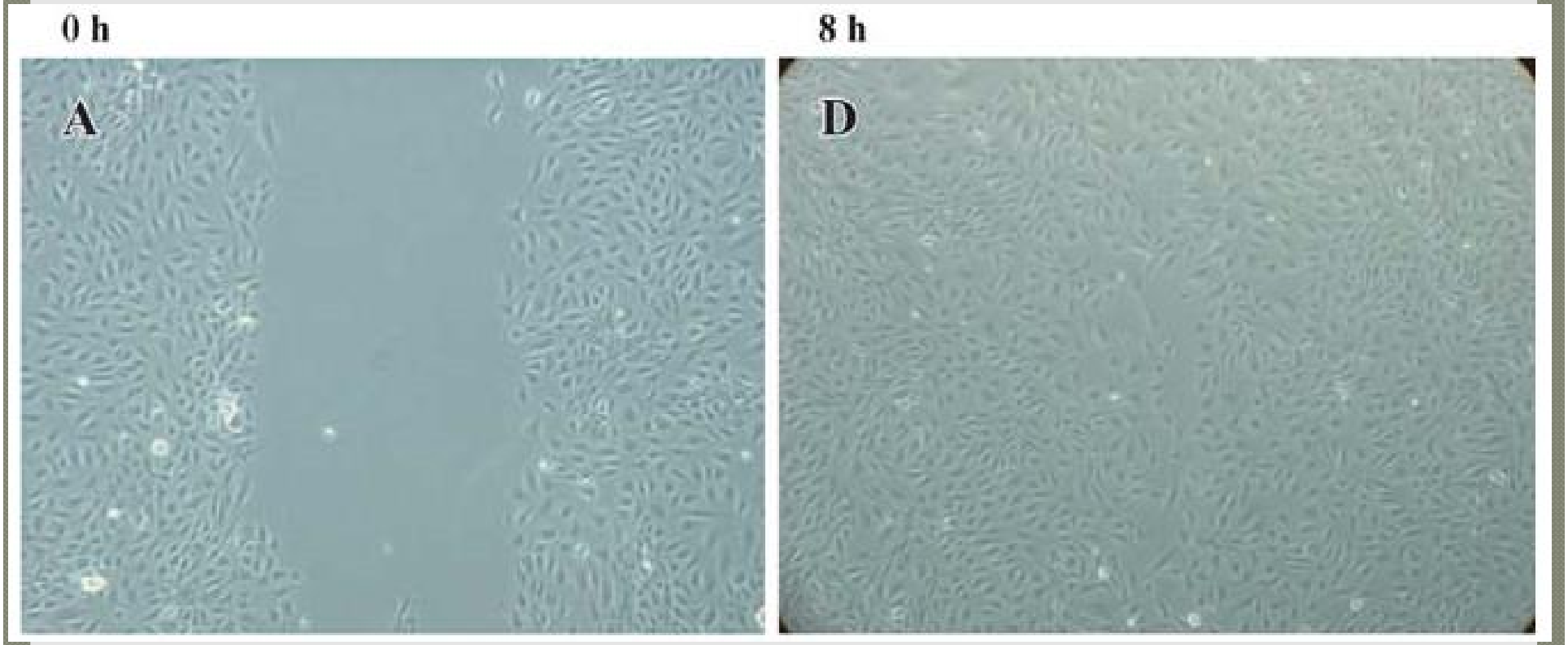
IC_{50} = 22 nM (KDR)

J. Med. Chem. 48, **2005**, 1610 - 1619.



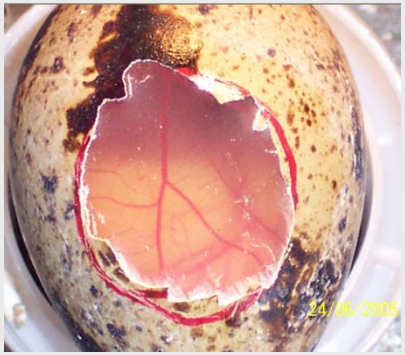
HUVEC

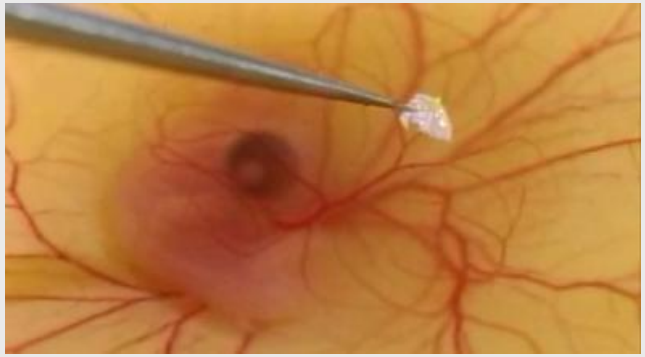
(Human Umbilical Endothelial Cells)

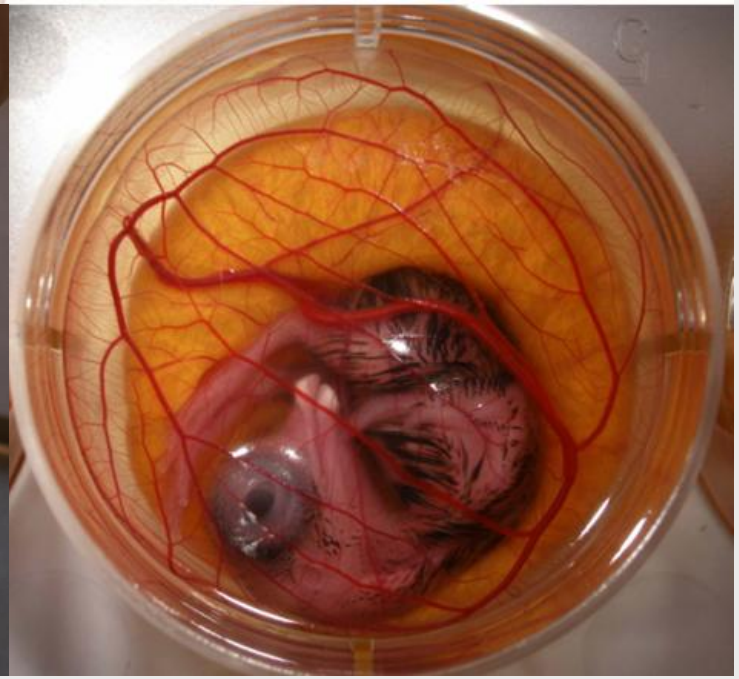


Predklinické testy (*in vivo*)

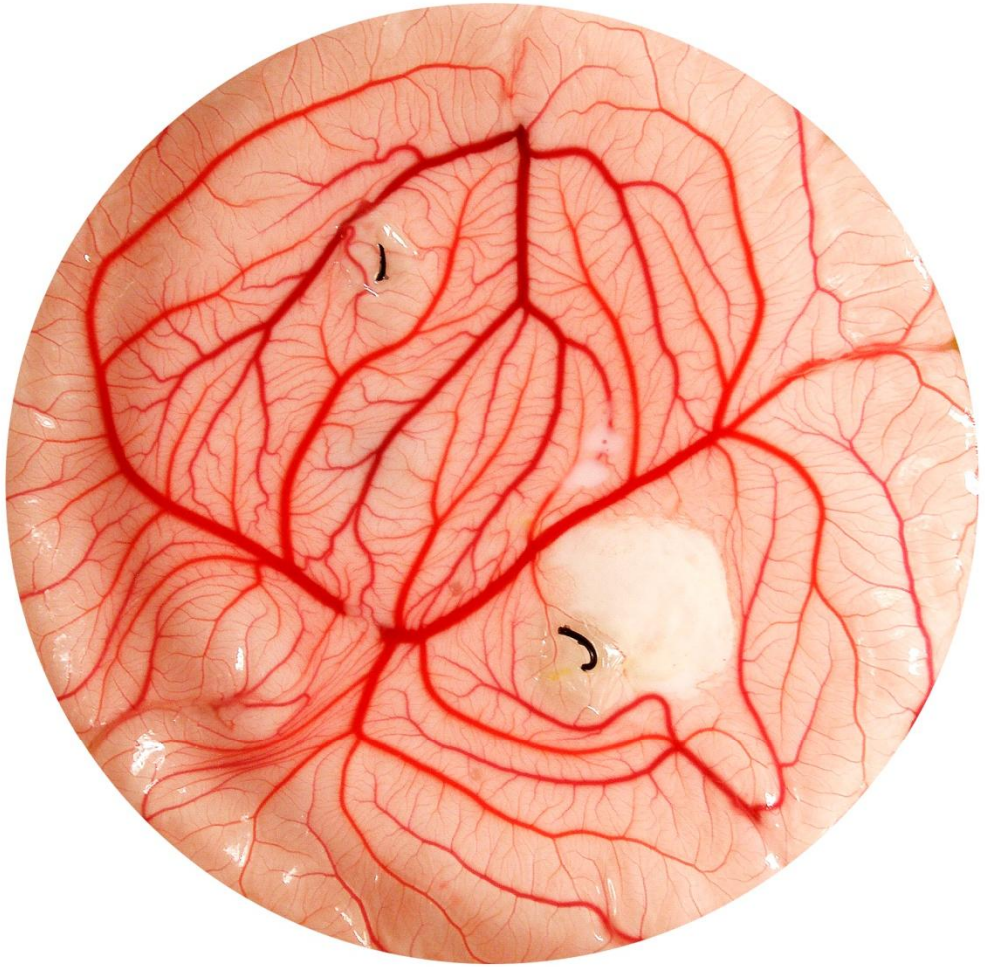
(Chick Chorioallantoic Membrane Model)



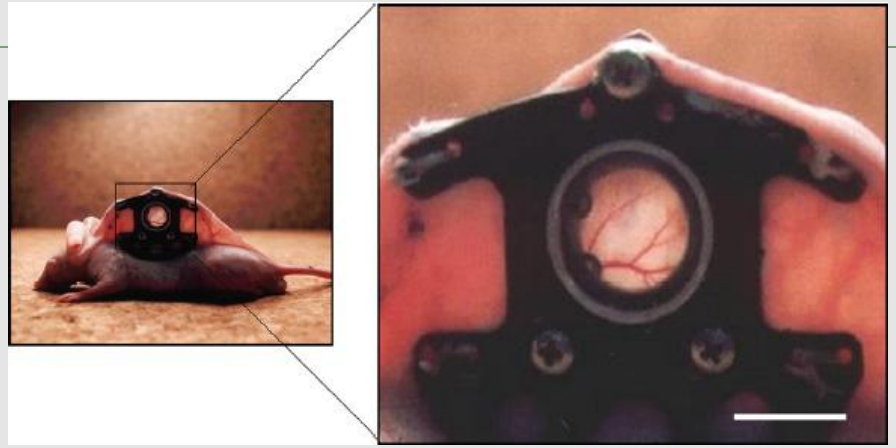




CAM



ž Dorsal skinfold window chamber model



ž Zebrafish embryo model

Zebra danio, Brachydanio rerio

Aequorea victoria

Súčasný stav antiangiogénnej liečby

- od 2004 zavedené 3 klinické liečivá
- kombinácia s chemoterapiou
- molekulové liečivá ⇨ nízka toxicita

- klinické pokusy ⇨ nové liečivá

AVASTIN® (Bevacizumab)

Humanised monoclonal VEGF antibody
FDA / 26.2.2004
Genentech, Roche

metastatic colorectal CCC, lung ca NSCLC, and breast cancer BC

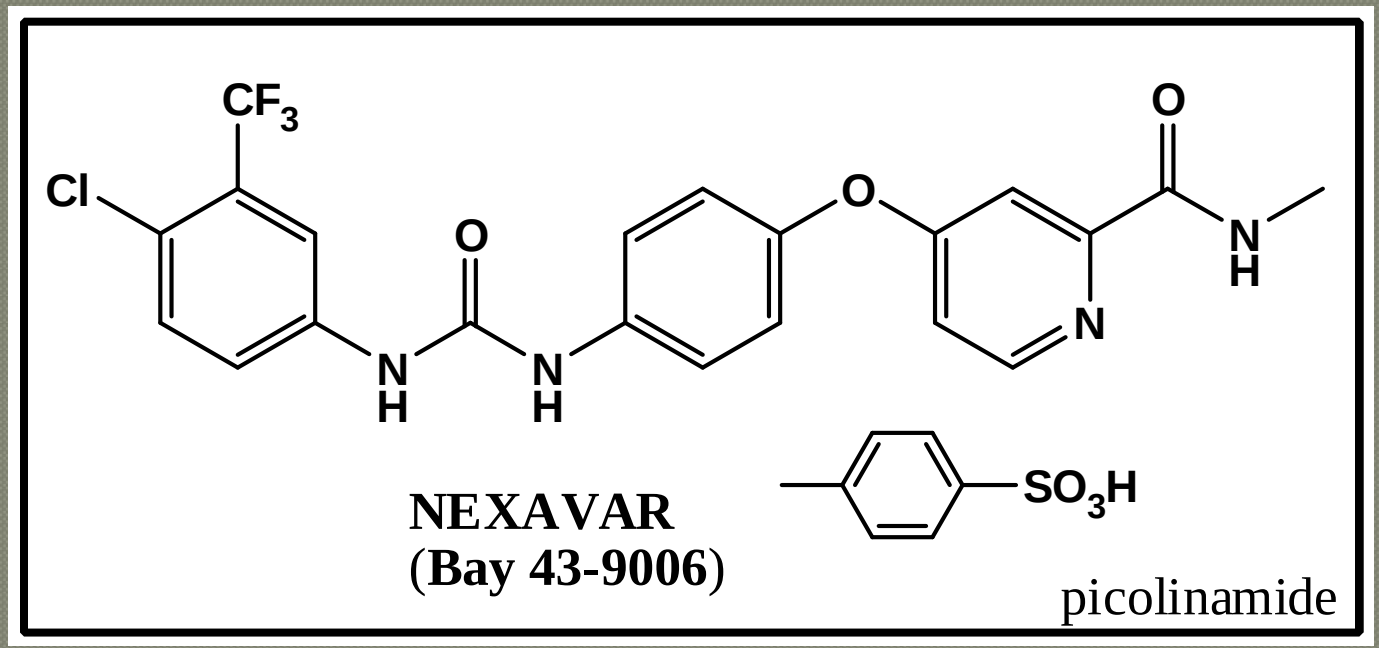
4 230 Euro / g

121 Euro / day

[1] Hurwitz H. et al. *N Engl J Med* 350, 2004, 2335-42.

NEXAVAR® (Sorafenib tosylate)

- FDA 20.12.2005, Bayer/Onyx
- advanced renal cell ca (RCC)



NEXAVAR
(Bay 43-9006)

picolinamide

212 Euro/g

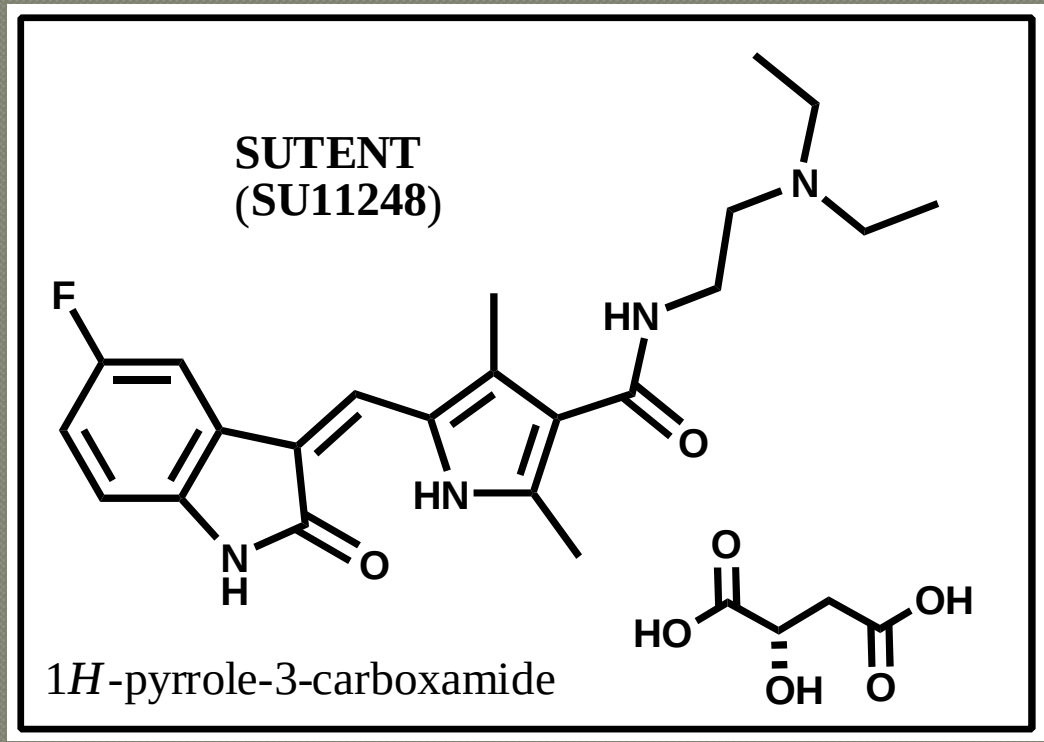
170 Euro/day

SUTENT® (Sunitinib L-malate)

- FDA 26.1.2006 , Pfizer, Inc.
  GIST and advanced renal cell carcinoma
- VEGFR multi TK inhibitor oral drug

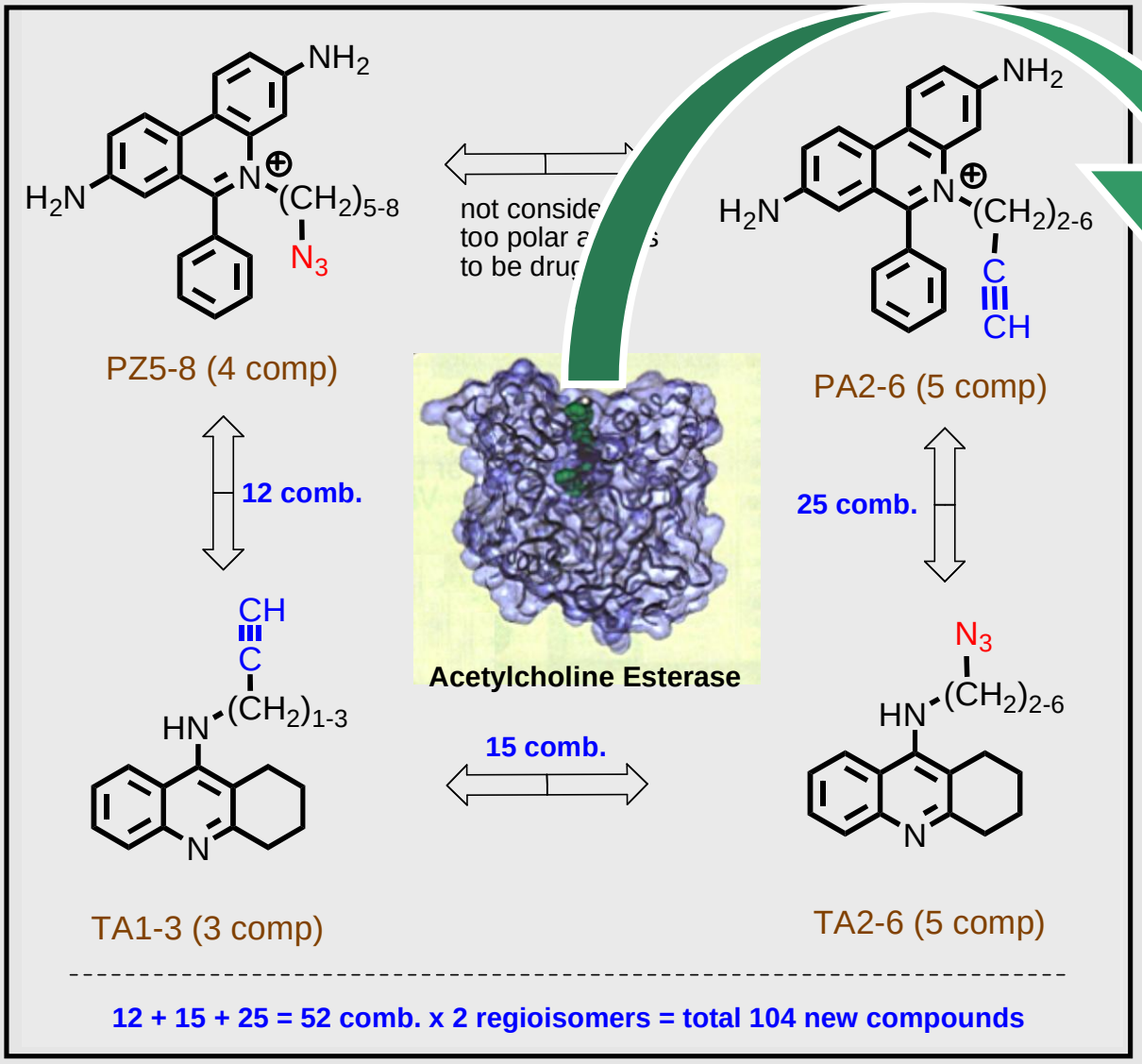
4 437 Euro / g

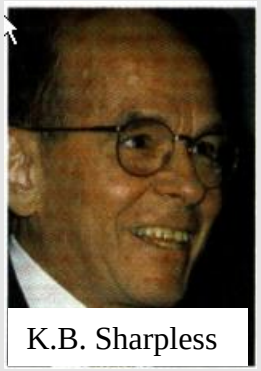
151 Euro / day



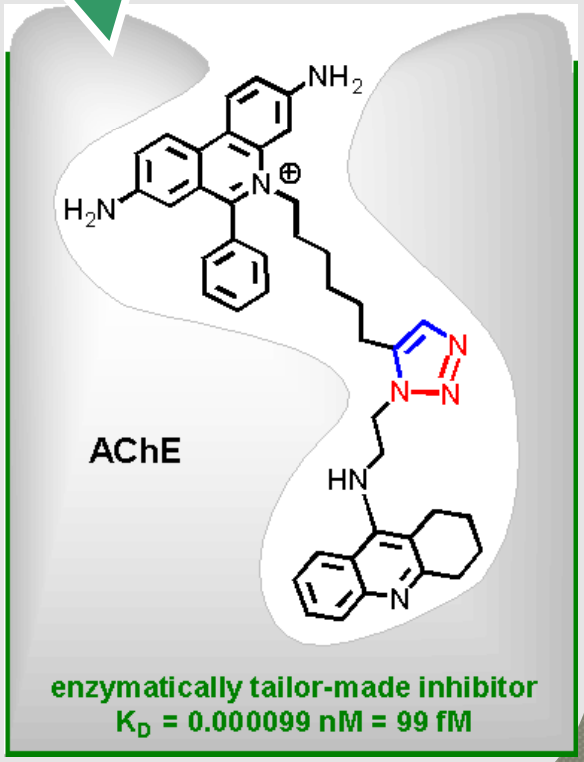
Orthogonal In Situ Click Chemistry

The enzyme AChE catalyzes the formation of its own femtomolar inhibitor.





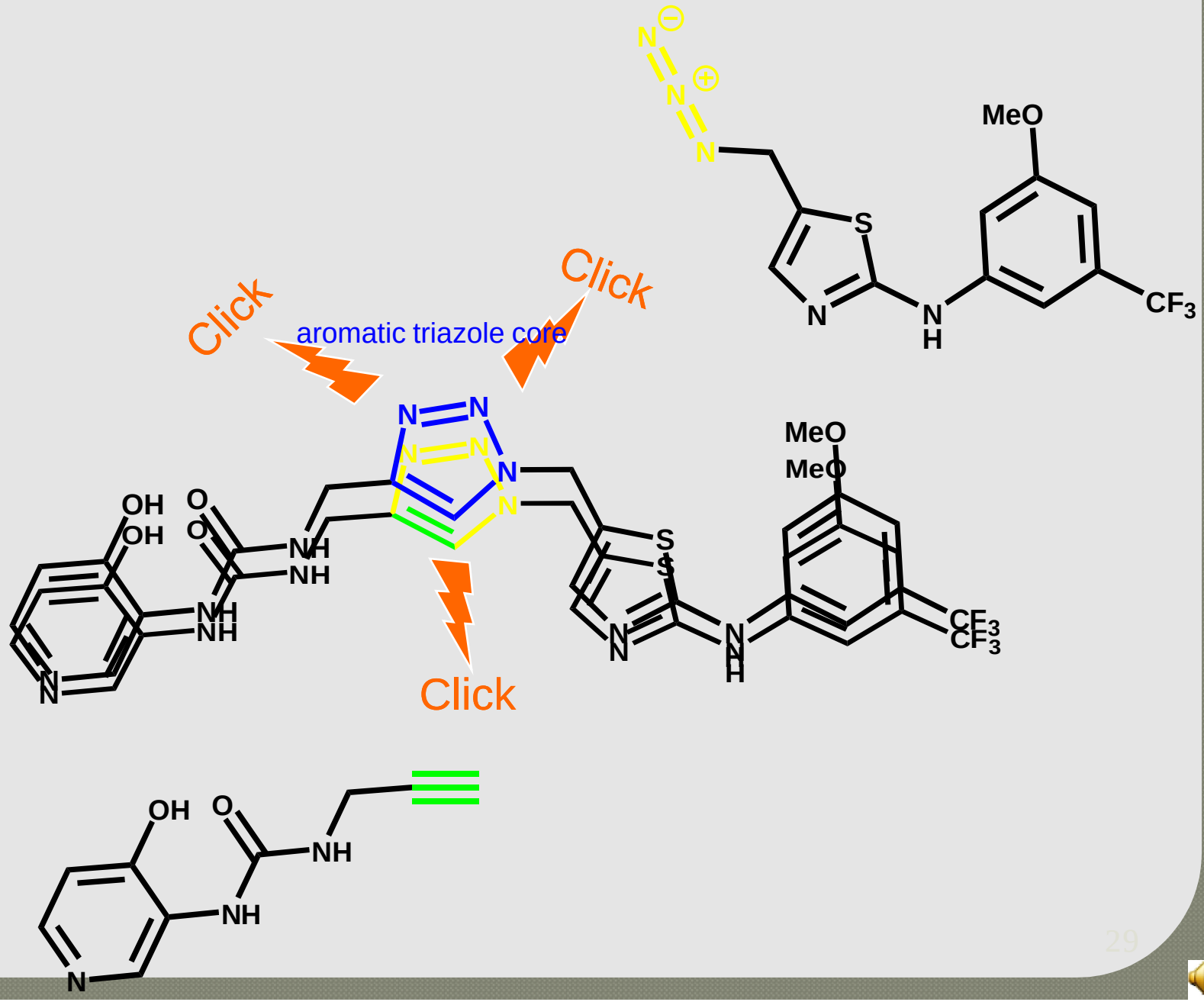
K.B. Sharpless



Sharpless et al. *Angew. Chem. IE* 41 2002 1053-1057.

JACS 2004, 126, 12809 - 12818

Irreversible Ligation by Click [3+2] Cycloaddition



COST CM 0602 (2007-2011)

Inhibitors of angiogenesis: design, synthesis and biological exploitation

http://www.cost.esf.org

www.angiokem.org

Ireland
France
Germany
Belgium
Switzerland
Poland
Norway
Spain
Greece
Czech Rep.
England
Slovakia Rep.
Sweden
Turkey
Italy

Denmark, UK, Israel, Cyprus, Serbia, Slovenia

Ďakujem za pozornosť

NATURE IS EXCITING

biological assays

- ***In vitro*** / cultured EC
  - proliferation (MTT), migration (Boyden chamber, Wound healing), tube formation models (differentiation, Matrix assay)
  - culture organ model (Rat aortic ring assay, mimics *in vivo* model, different cells + extracellular matrix, no tumor cells)
- ***In vivo*** (complexity, biological)

  pericytes, smooth muscle cells, fibroblasts, extracellular matrix, mesenchymal cells, immune cells, tumor cells
  - Subcutaneous model (sponge / Matrigel + cells/ inhibitor —> amount of haemoglobin)
  - Corneal angiogenic assay
  - Dorsal air sac model (ca cells in chamber with f
  - Different tumor models (xenograft / imunodef. survival, tumor cells produce GFP, vessels are dark)