Les trésors de la nature: les composés naturels en tant qu'inhibiteurs des principales caractéristiques du cancer









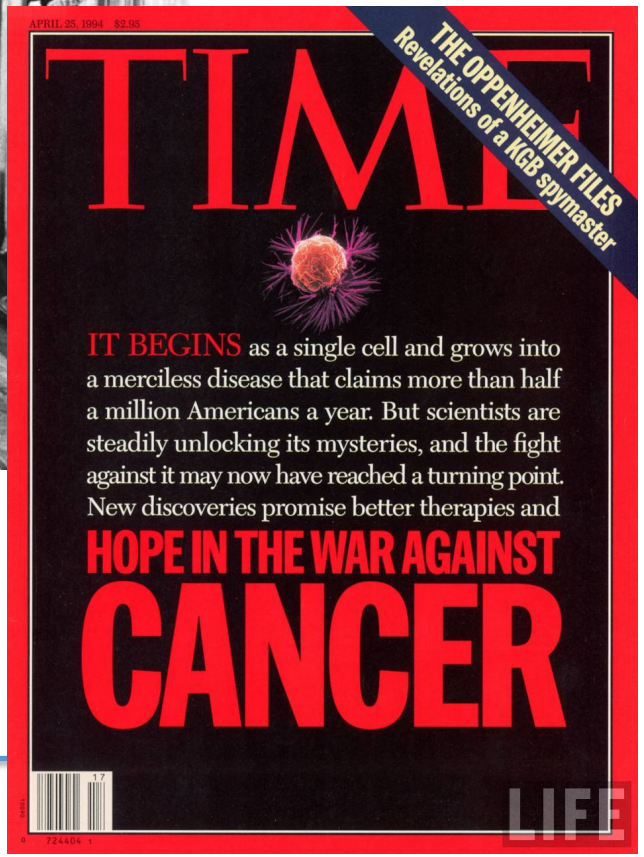

Fondation de "Recherche Cancer et Sang"
Hôpital Kirchberg – Luxembourg

marc.diederich@lbmcc.lu *http://www.lbmcc.lu*

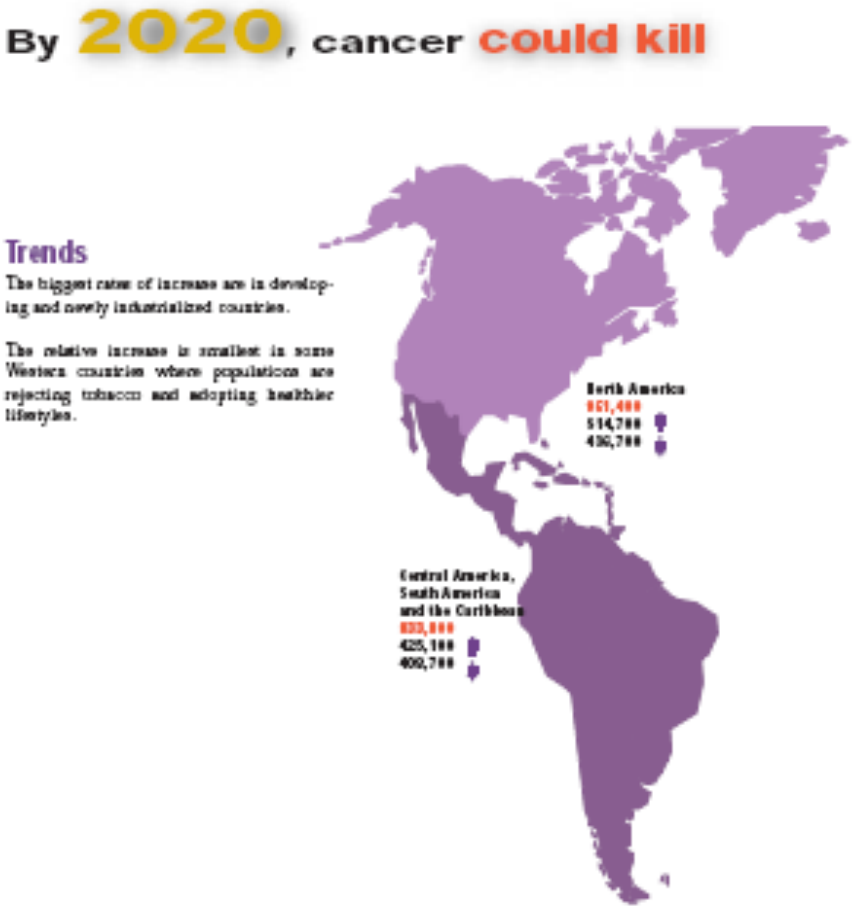
"I will also ask for an appropriation of an extra $100 million to launch an intensive campaign to find a cure for cancer, and I will ask later for whatever additional funds can effectively be used. The time has come in America when the same kind of concentrated effort that split the atom and took man to the moon should be turned toward conquering this dread disease. Let us make a total national commitment to achieve this goal."

President Nixon - January 1971 State of the Union address

President Richard Nixon signing the National Cancer Act on December 23, 1971. Courtesy of NCI.



Every year about seven million people die from cancer, making this disease responsible for 12.5% of deaths worldwide (Coseri S, 2009)



Why We're Losing The War On Cancer!

And How To Win It?

By Clifton Leaf Additional Reporting Doris Burke
March 22, 2004 (FORTUNE Magazine)

Avastin, Erbitux, Gleevec ... The new wonder drugs might make you think we're finally beating this dreaded scourge. We're not.

http://money.cnn.com/magazines/fortune/fortune_archive/2004/03/22/365076/index.htm

Why natural products are good Source of anticancer drugs?

Almost 74% (48/65) of all drugs approved either were natural products, were based thereon, or mimicked them in one form or another (1981-2002)

Newman DJ, Cragg GM, and Snader KM.,
J. Nat. Prod., 2003, 66, 1022-1037.

Slide from: Bharat Aggarwal

Pacific yew (*Taxus brevifolia*)	Taxol
Rosy periwinkle (*Catharanthus roseus*)	Vinblastin and vincristine
Foxglove (*Digitalis purpurea*)	Digitalis
Meadowsweet (*Spiraea alba*)	Aspirin

Taxus brevifolia

Catharanthus roseus

Digitalis purpurea

Spiraea alba

Hallmarks of Cancer: The Next Generation

Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]
[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland
[2]The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA
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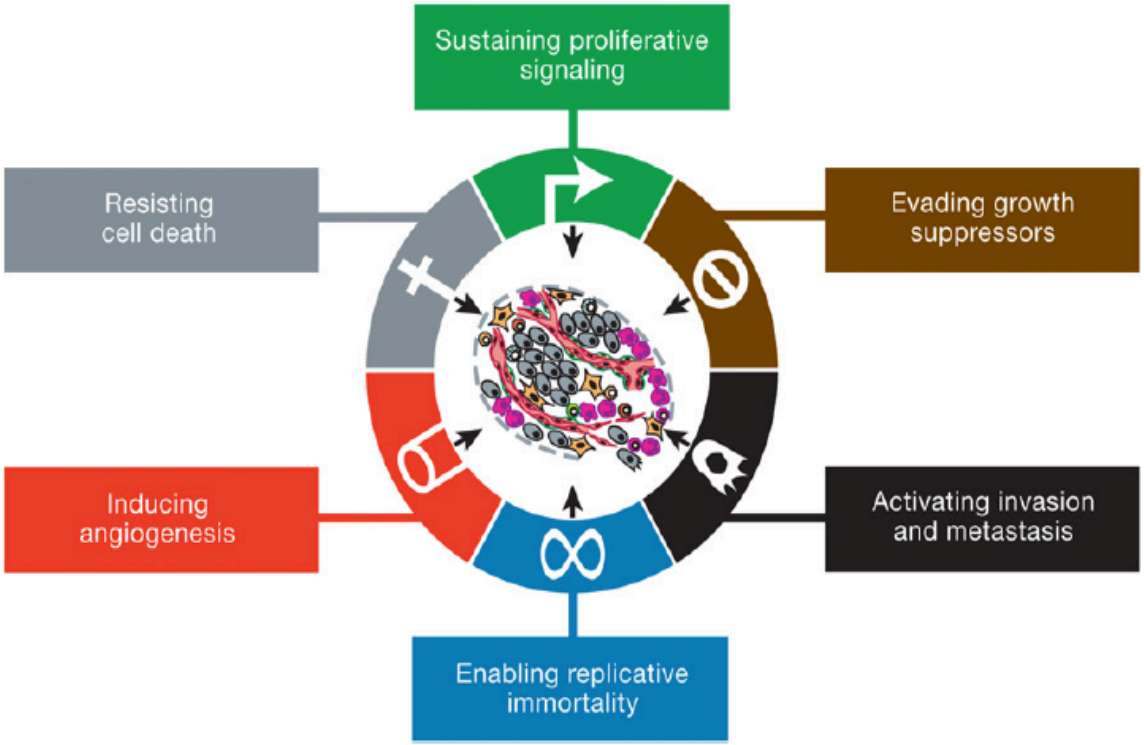
*Correspondence: dh@epfl.ch (D.H.), weinberg@wi.mit.edu (R.A.W.)
DOI 10.1016/j.cell.2011.02.013



Hallmarks of Cancer: The Next Generation

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[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland
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*Correspondence: dh@epfl.ch (D.H.), weinberg@wi.mit.edu (R.A.W.)

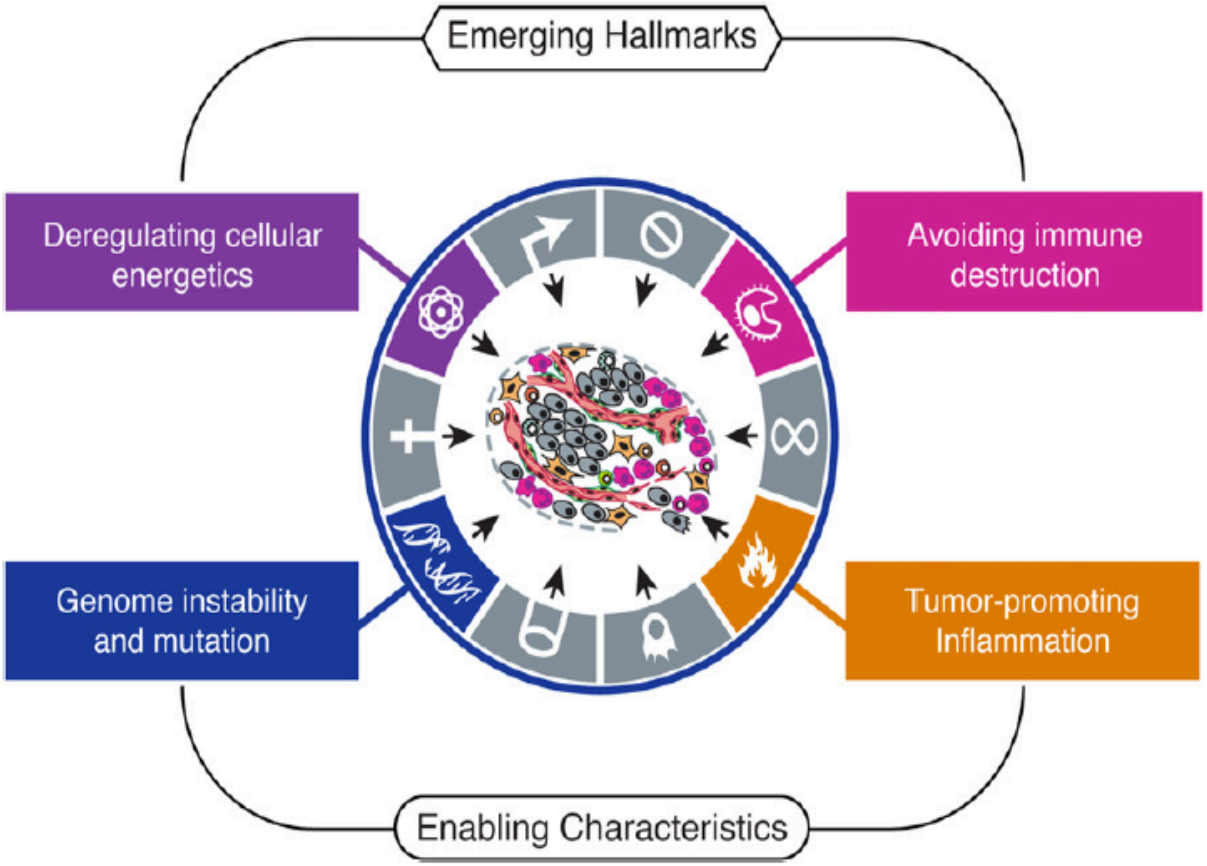


Figure 3. Emerging Hallmarks and Enabling Characteristics

An increasing body of research suggests that two additional hallmarks of cancer are involved in the pathogenesis of some and perhaps all cancers. One involves the capability to modify, or reprogram, cellular metabolism in order to most effectively support neoplastic proliferation. The second allows cancer cells to evade immunological destruction, in particular by T and B lymphocytes, macrophages, and natural killer cells. Because neither capability is yet generalized and fully validated, they are labeled as emerging hallmarks. Additionally, two consequential characteristics of neoplasia facilitate acquisition of both core and emerging hallmarks. Genomic instability and thus mutability endow cancer cells with genetic alterations that drive tumor progression. Inflammation by innate immune cells designed to fight infections and heal wounds can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumor-promoting consequences of inflammatory responses.

Enabling characteristic: Inflammation

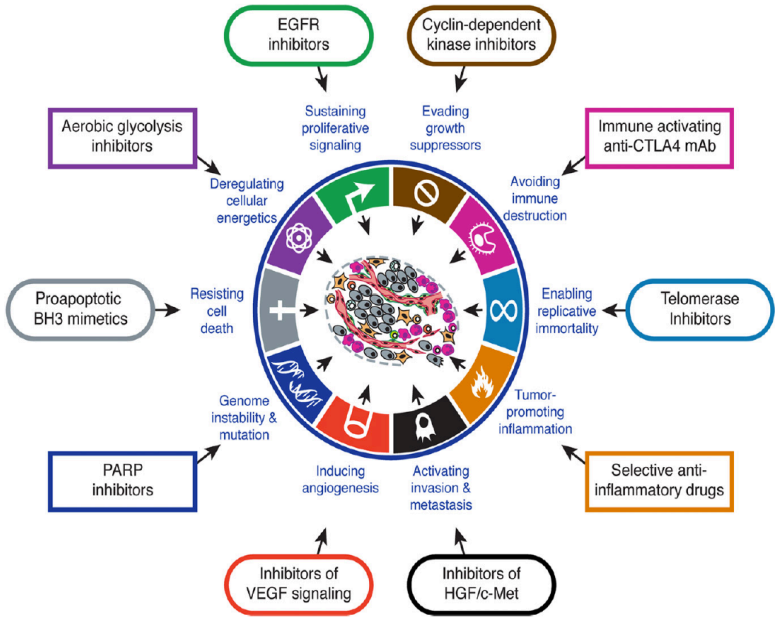
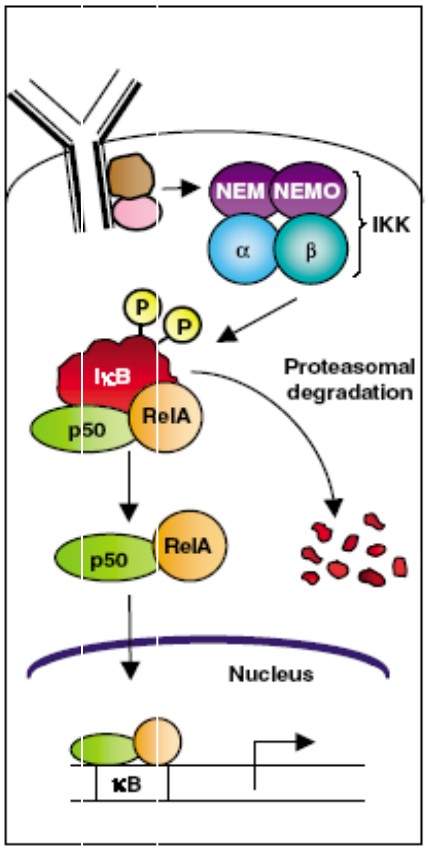


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

TIME Feb. 23, 2004

NF-κB: two main signaling pathways

Canonical pathway



(Gilmore, *Oncogene* 2006)

The canonical NF-κB activation pathway

- Typically **triggered through TNFR, IL-1R or TLR**
- Recruitment and activation of the classical **IKK** complex which phosphorylates IκBα leading to ubiquitylation and degradation via the proteasome pathway
- The free **RelA(p65)/p50** migrates to the nucleus where it activates target genes involved in immune response.

TNFR: TNF receptor, IL1R: IL-1 receptor, TLR: Toll Like Receptor, IKK: IκB kinase

NF-κB and cancer

NF-kB target genes related to the enhancement of tumor progression

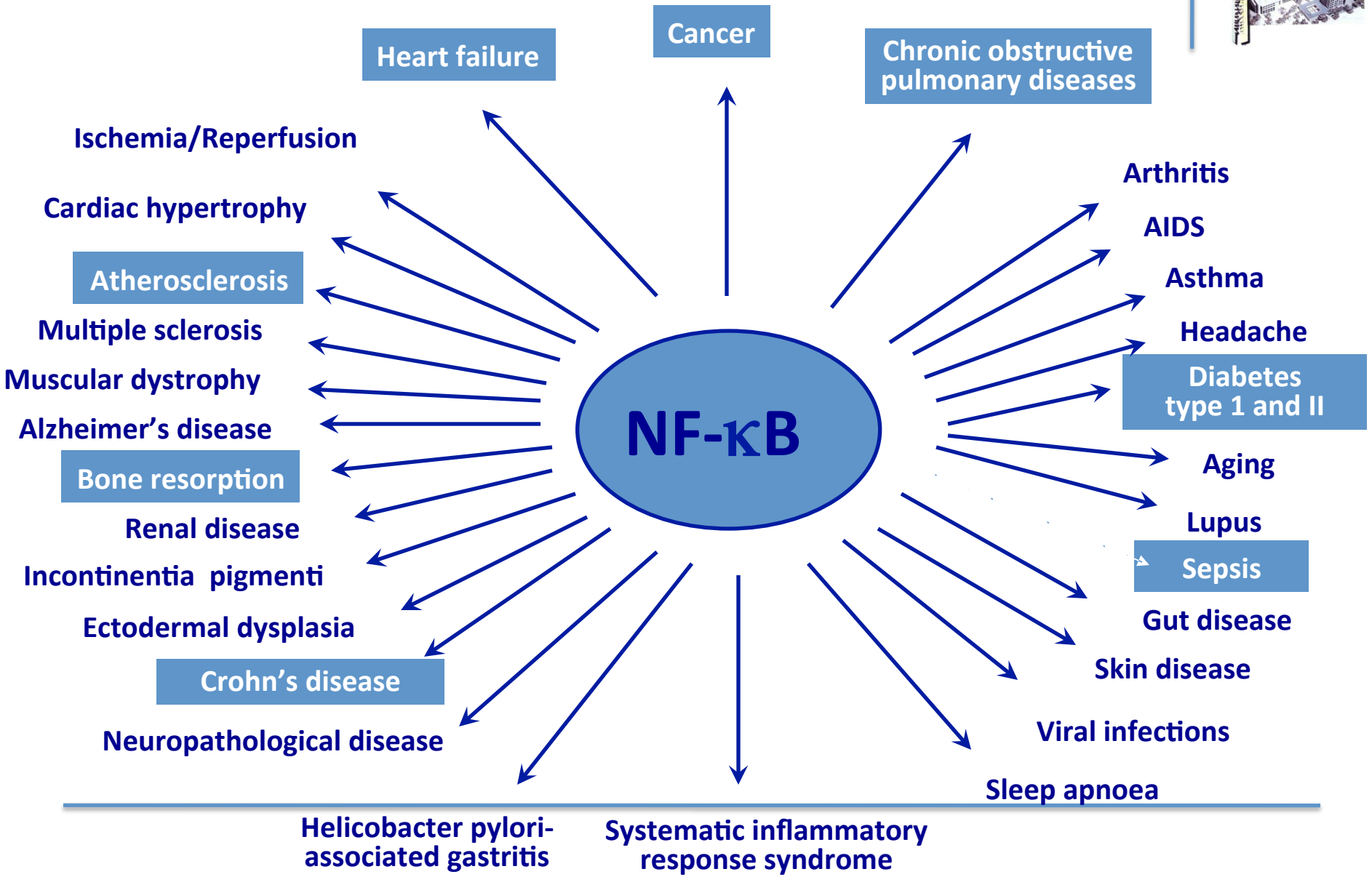
Activity	Genes
Inflammation	TNF, IL-1, chemokines
Cellular immortality	telomerase
Cell survival	Bcl-xl, cIAP, XIAP, cFLIP
Angiogenesis	VEGF, TNF, IL-1, IL-8
Proliferation	TNF, IL-1, IL-6, cyclin D1, c-myc
Tumor promotion	Cox-2, iNOS, MMP-9
Metastasis	ICAM-1, V-CAM, ELAM-1

(Nishikori et al. 2005)

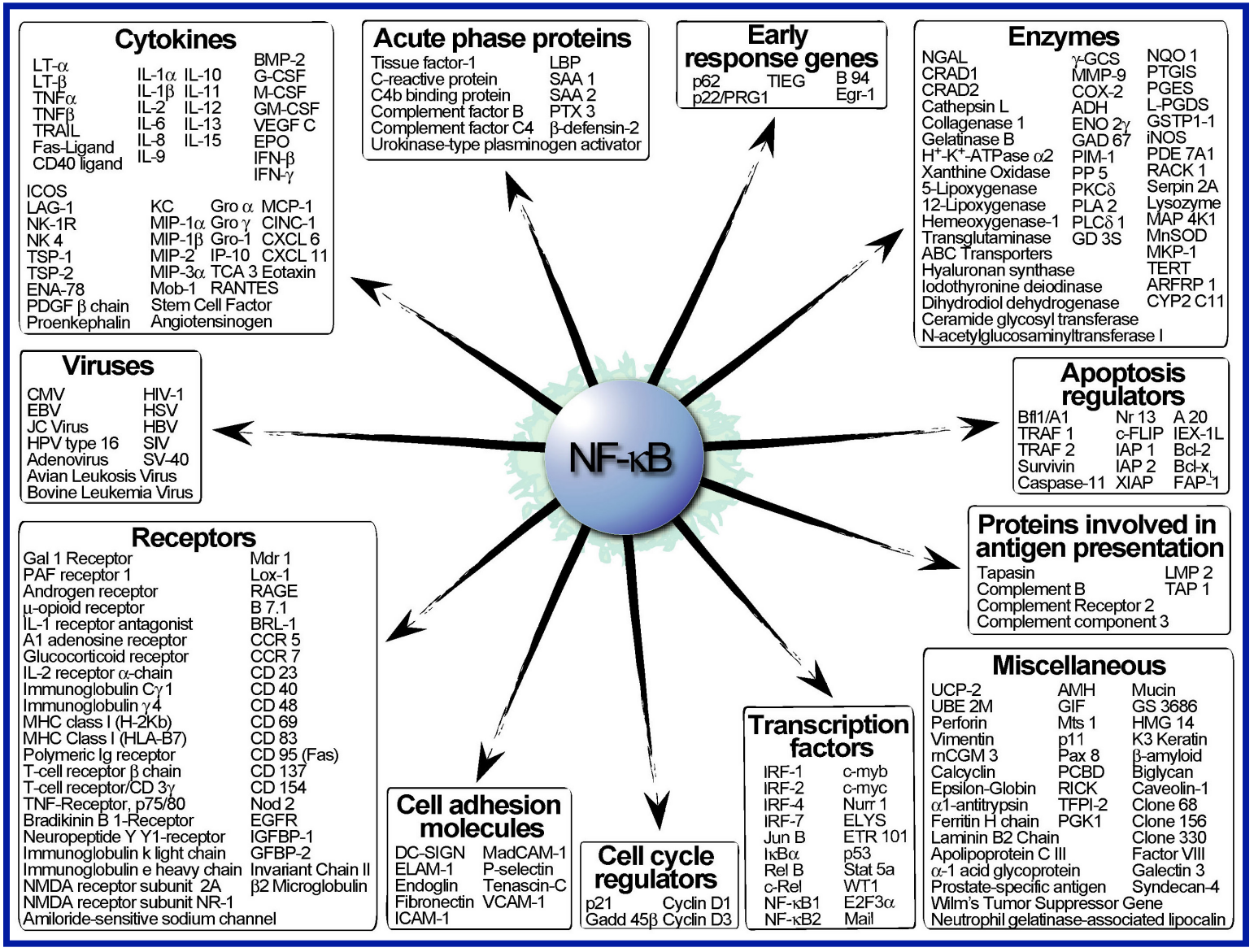
- In addition to its role in immune and inflammatory responses, NF-κB also plays a pivotal role in the generation and maintenance of malignancies
- NF-κB has been considered as a target for cancer drug development
- Inhibitors of NF-κB activation are extensively sought.

cIAP, cellular inhibitors of apoptosis : XIAP, X chromosome-linked inhibitors of apoptosis : cFLIP, caspase 8-FASassociated death domain (FADD)-like IL-1b-converting enzyme inhibitory protein : VEGF, vascular endothelial growth factor : COX2, cyclooxygenase 2 : iNOS, inducible nitric oxide synthase : MMP-9, metalloproteinase-9 ICAM-1, intracellular adhesion molecule-1 : VCAM-1, vascular cell adhesion molecule-1 : ELAM-1, endothelial-leukocyte adhesion molecule-1.

NF-κB has been linked to several diseases



NF-κB -regulated genes



Kumar A, Takada Y, Boriek AM, Aggarwal BB. *Journal of Molecular Medicine* 2004;82:434-48.

Role of inflammation in tumorigenesis

Notch-1
PPAR-γ
STAT3
β-catenin
p53
AP-1

NF-κB

DNA damage
Oncogenes

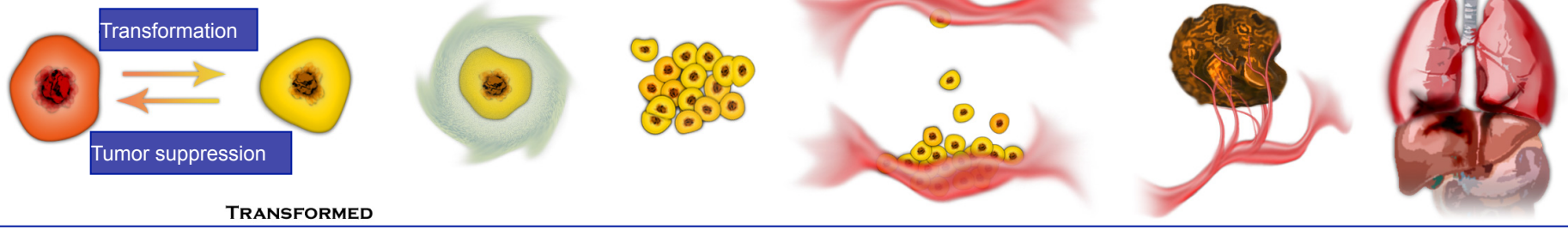
Bcl-xl
Bcl-2
Survivin
C-FLIP
cIAP-1
cIAP-2
XIAP

Cyclin D1
C-myc
TNF
IL-1
IL-6
COX2

MMP-9
uPA
ICAM-1
ELAM-1
VCAM-1

VEGF

CXCR4
TWIST



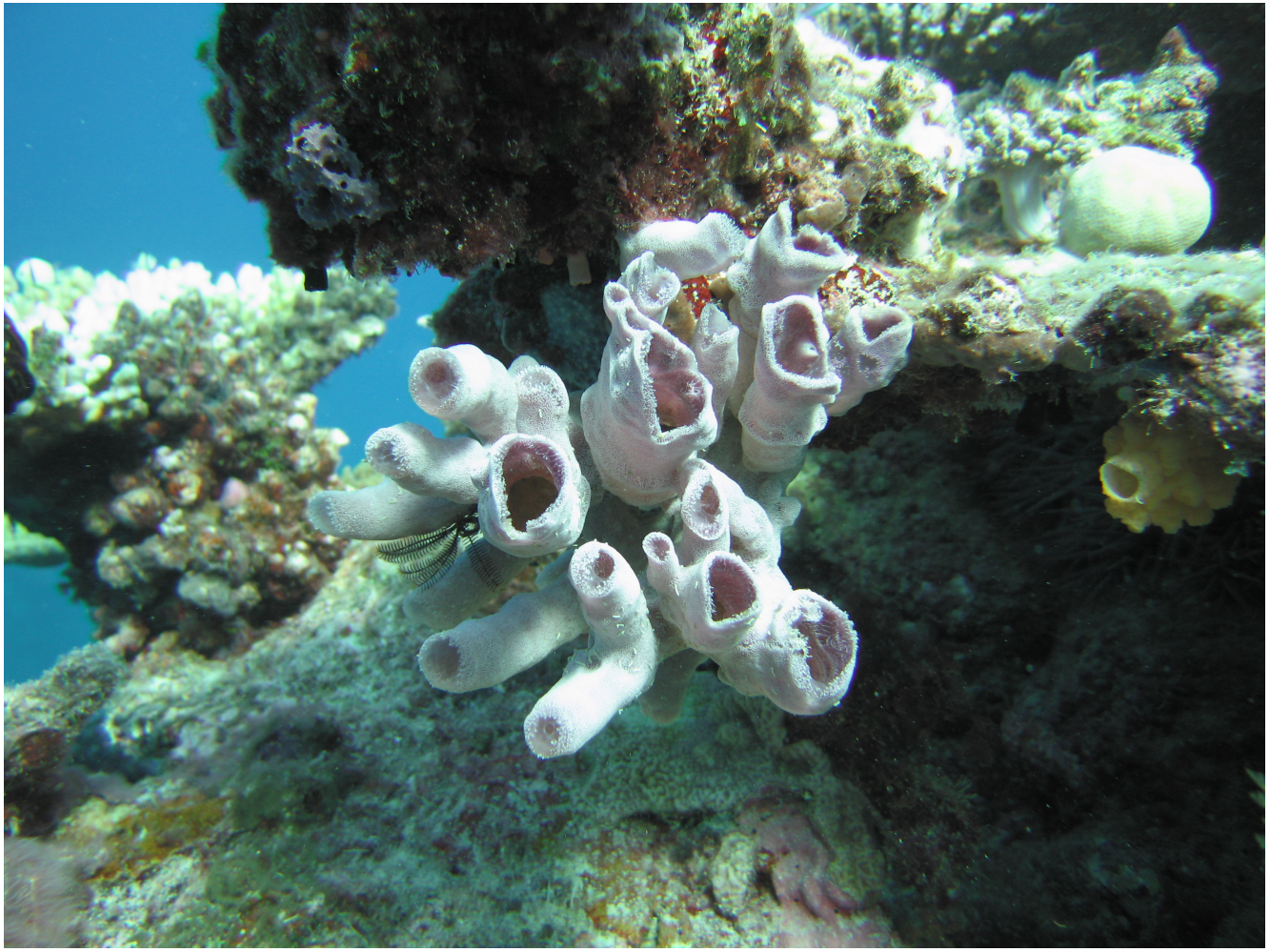
Normal cell → Transformation → Survival → Proliferation → Invasion → Angiogenesis → Metastasis

10-20 Years

10 Years

Inflammation

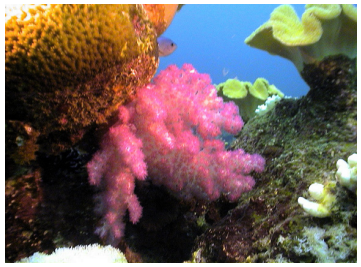
At this very moment, the cure for cancer may be growing somewhere ….




29%
71%

NF-kB screening activity

- marine invertebrate extracts from the NCI (USA) and from Fiji



Biochemical Pharmacology 78 (2009) 592–606

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journal homepage: www.elsevier.com/locate/biochempharm

The inhibition of TNF-α-induced NF-κB activation by marine natural products

Florence Folmer[a], Marcel Jaspars[a], Godofredo Solano[a], Silvia Cristofanon[b], Estelle Henry[b], Jioji Tabudravu[a], Kenny Black[c], David H. Green[c], Frithjof C. Küpper[c], William Aalbersberg[d], Klaus Feussner[d], Mario Dicato[b], Marc Diederich[b,*]

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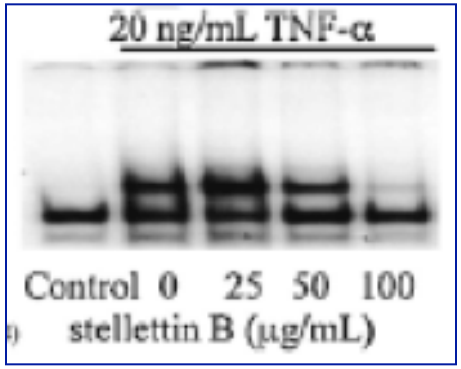
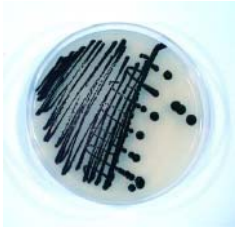
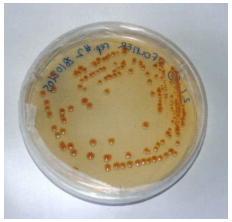
[d] Institute of Applied Sciences, Faculty of Science and Technology, University of the South Pacific, Private Mail Bag, Suva, Fiji



← NF-kB

- bacterial strains from marine sediment





- algal strains from the Culture Collection of Algae and Protozoa (Scottish Association for Marine Sciences)

The biotic and abiotic factors governing the marine ecosystem

Abiotic factors

Biotic factors

salinity, extreme pH levels

UV radiation

parasitism

fouling

exposure to air, hypoxia/oxidative stress

predation (incl. grazing)

extreme temperatures

competition for food

currents, turbulence, and pressure

lack of sunlight

competition for space

Folmer F., Schumacher M., Jaspars M., Dicato M., Diederich M. in Chemical ecology and medicinal chemistry of marine NF-κB inhibitors, 2008

106 *J. Nat. Prod.* **2008,** *71,* 106–111

NF-KB-Inhibiting Naphthopyrones from the Fijian Echinoderm *Comanthus parWicirrus*

Florence Folmer,[†] William T. A. Harrison,[†] Jioji N. Tabudravu,[†] Marcel Jaspars,[*,†] William Aalbersberg,[‡] Klaus Feussner,[‡] Anthony D. Wright,[§] Mario Dicato,[⊥] and Marc Diederich[⊥]

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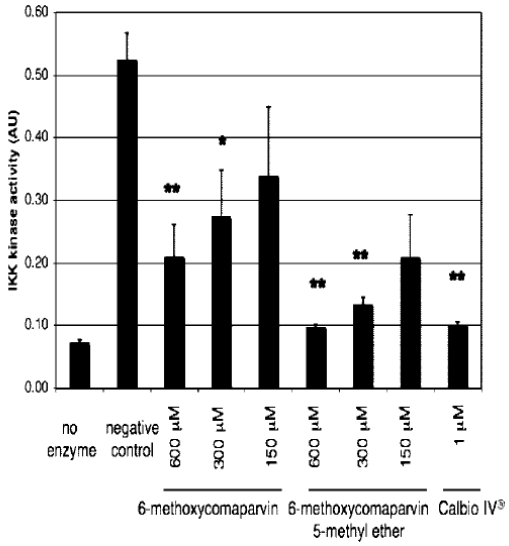
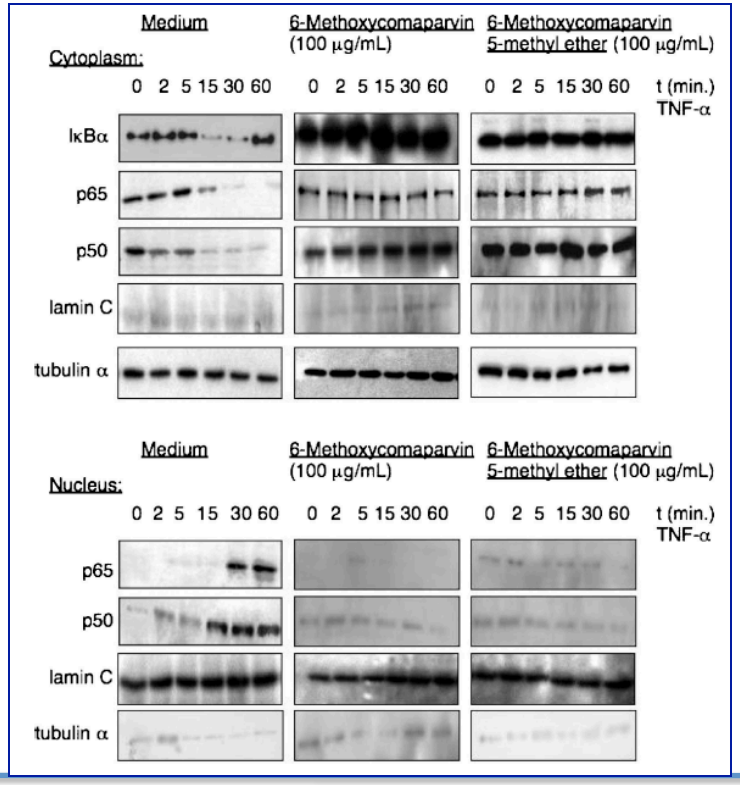
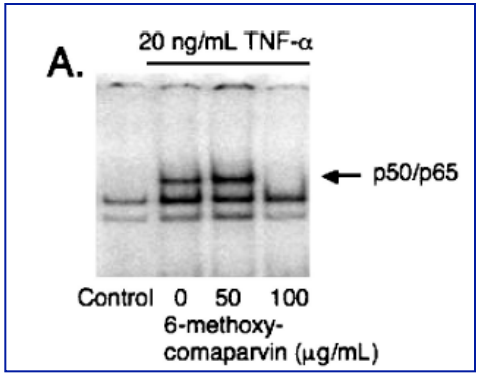
ReceiVed June 18, 2007



Inhibition of IKK kinase activity

Inhibition of Ikb degradation and NF-kB translocation





Inhibition of NF-kB binding

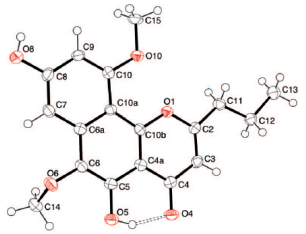


Figure 1. View of the C2 molecule of 6-methoxycomaparvin (**1**)·3/2 H_2O, showing 50% displacement ellipsoids. H atoms are represented by arbitrary spheres. The intramolecular H bond is indicated by a dashed line.

Elucidation of NF-kB cell signaling pathways

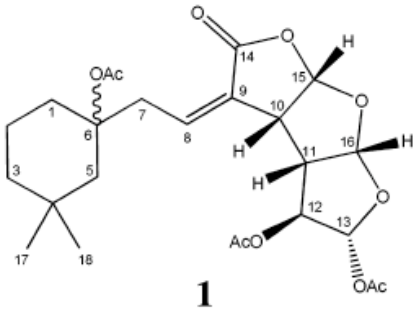
J. Nat. Prod. **2009,** *72,* 1471–1476

Bioactive Diterpene Derivatives from the Marine Sponge *Spongionella* sp.

Mostafa E. Rateb,[†,‡] Wael E. Houssen,[§] Marc Schumacher,[⊥] William T. A. Harrison,[∥] Marc Diederich,[⊥] Rainer Ebel,[†] and Marcel Jaspars*[,†]

Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, Meston Walk, Aberdeen AB24 3UE, Scotland, U.K., Institute of Medical Sciences, University of Aberdeen, Ashgrove Road West, Aberdeen AB25 2ZD, Scotland, U.K., Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg, 9 Rue Edward Steichen, L-2540 Luxembourg, Luxembourg, and Department of Chemistry, University of Aberdeen, Meston Walk, Aberdeen AB24 3UE, Scotland, U.K.

Received April 20, 2009



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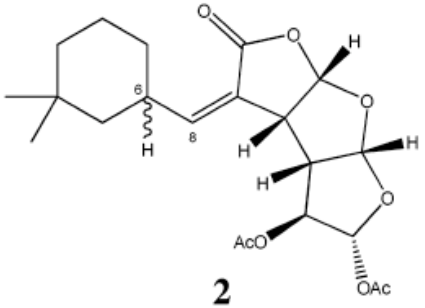

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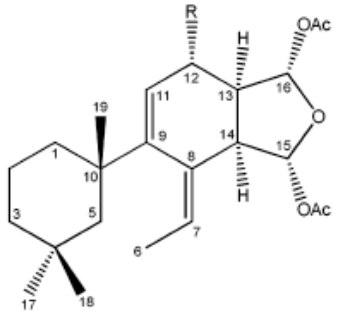
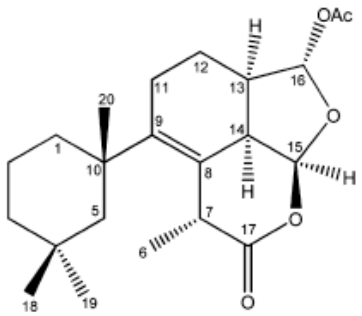


5, R = H
6, R = OH



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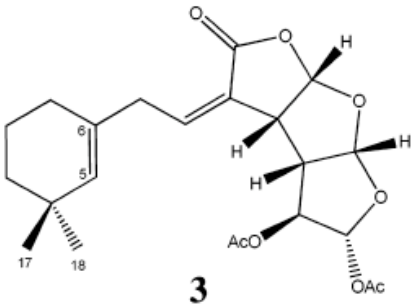

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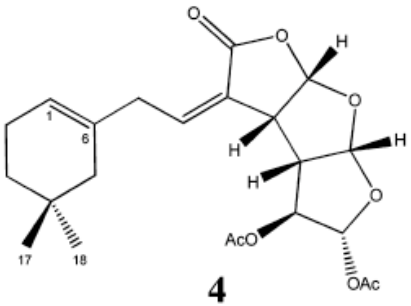

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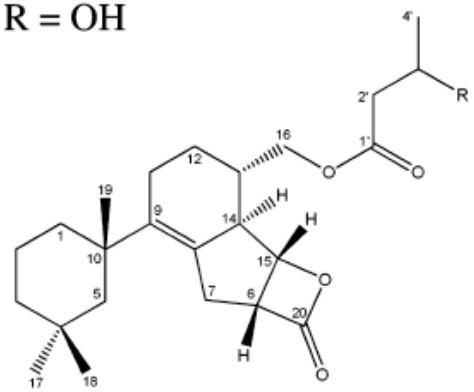
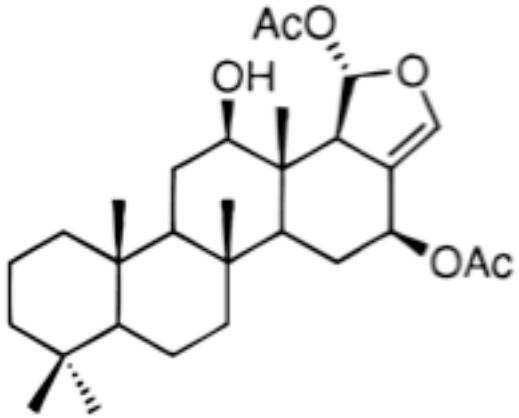


8, R = H
9, R = CH_3

gracilins (**1-6),** tetrahydroaplysulphurin-1 (**7),** 3′-norspongiolactone (**8),** spongiolactone (**9)**

Heteronemin isolated from *Porifera Hyrtios*



Heteronemin

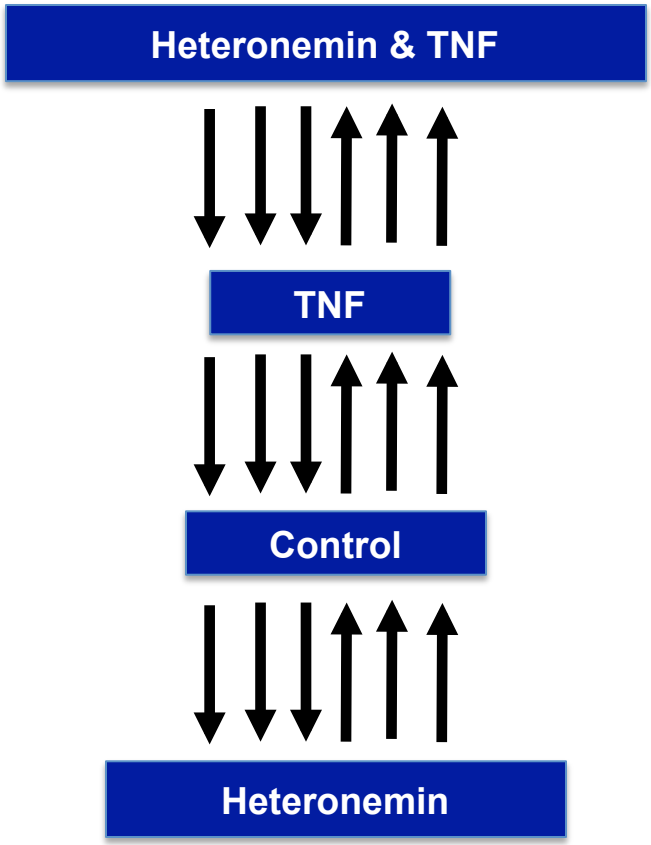
Heteronemin, first discovered from *Hyrtios erecta* by the research group of Wells and co-workers , was isolated from the Fijian sponge Porifera Hyrtios, it's a colourless oil soluble in methanol and DMSO.

This compound exhibited antimycobacterial activity against M. *tubercolosis* H37Rv with a MIC value of 6.25 mg/ml. An antitubercular activity was reported in litterature.

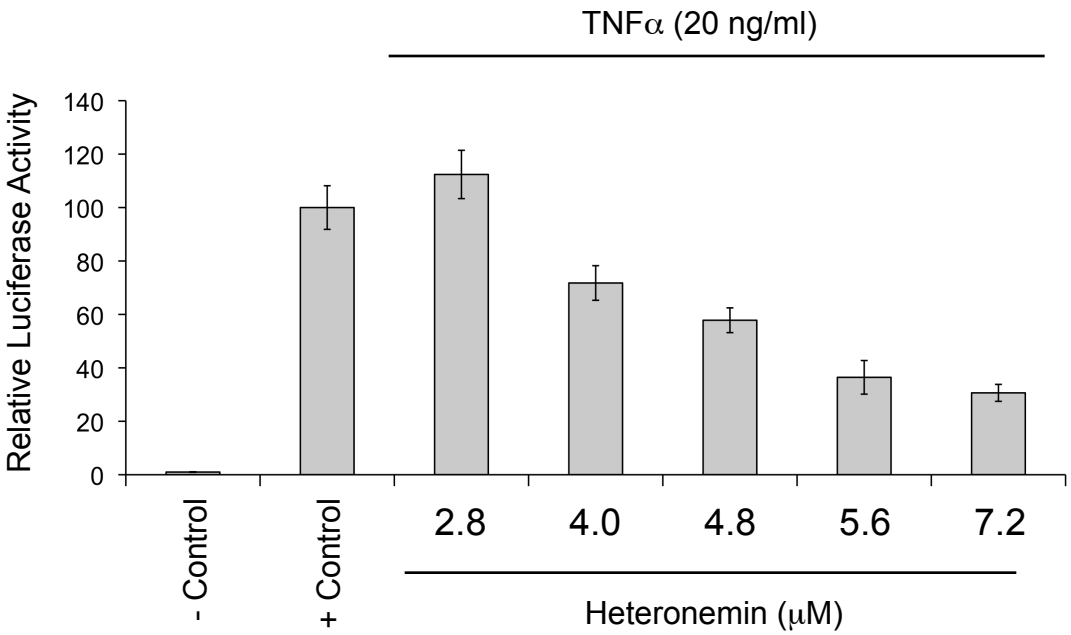
Heteronemin has been shown to be a Protein Farnesyl Transferase Inhibitor by Ledroit and co-workers.

Biologically relevant questions and experimental design

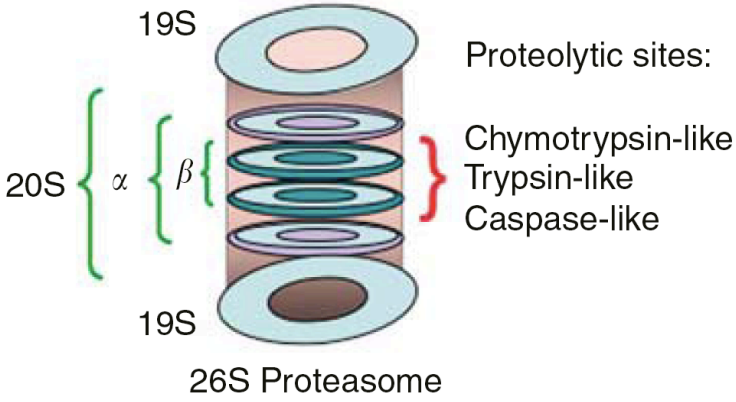
- Which genes respond differentially to Heteronemin stimulation?
- Which genes are differentially expressed by TNF-treatment?
- Which genes respond to Heteronemin stimulation in TNF-treated cells?
- Which genes display an interaction effect between both molecules (Heteronemin and TNF): synergistic or antagonistic

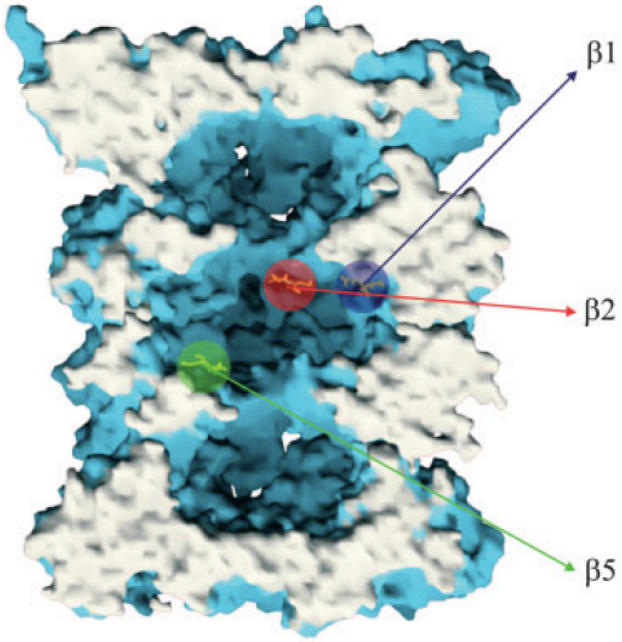


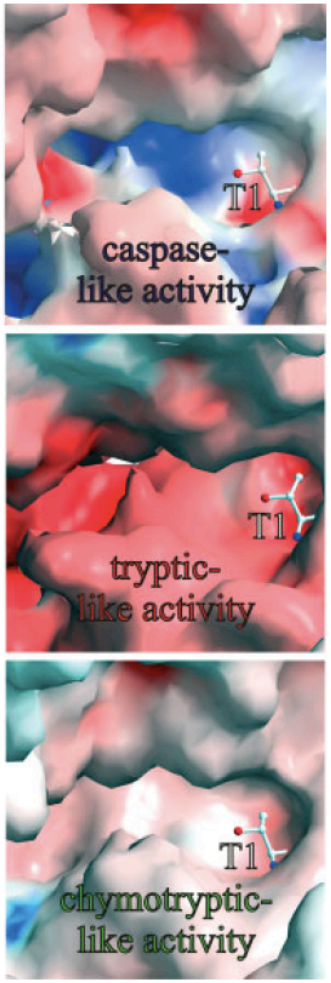
Inhibition of NF-κB transactivation



Heteronemin – proteasome

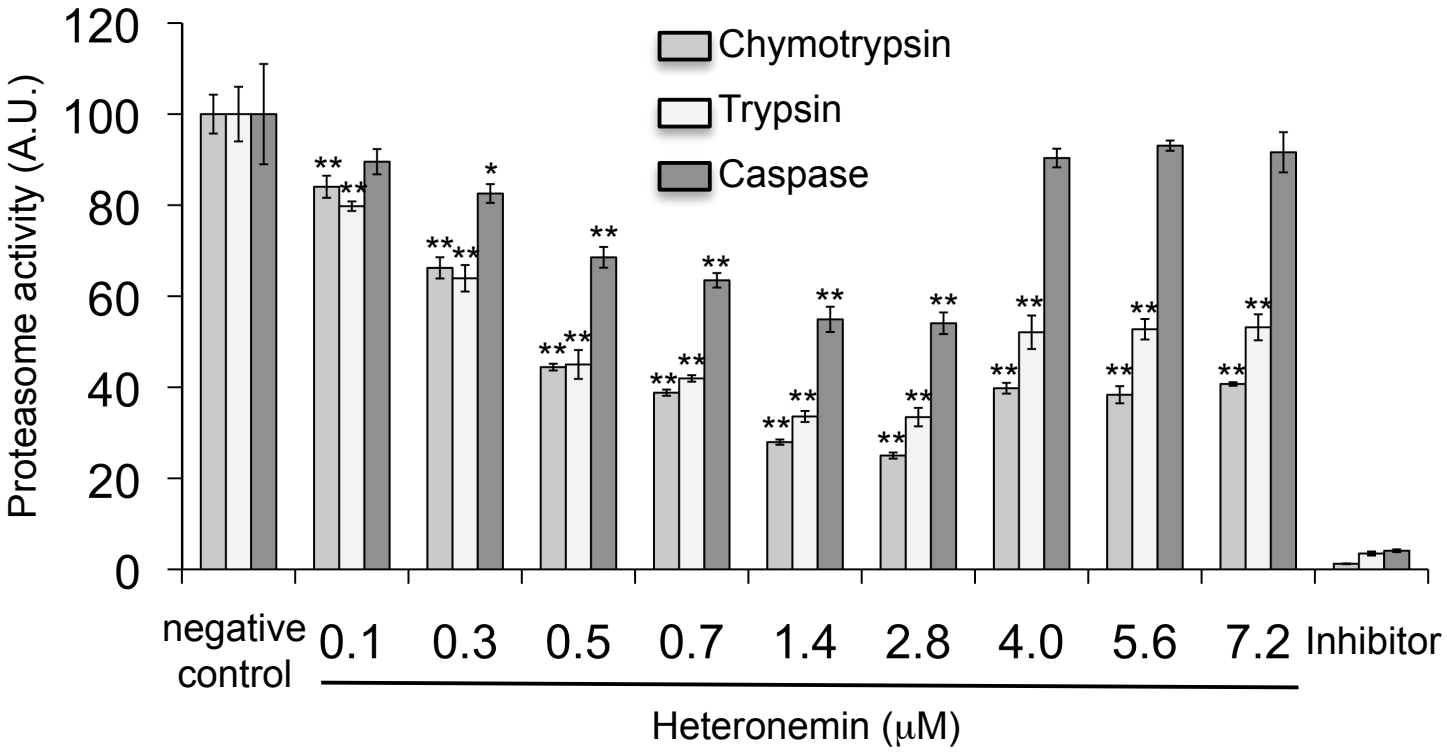






Chauhan D, Hideshima T, Anderson KC. Br J Cancer. 2006 Oct 23;95(8):961-5
Groll M, Huber R, Moroder L. J Pept Sci. 2009 Feb;15(2):58-66

Inhibition of proteasome activity



Hallmark: Resisting Cell death

Apoptosis

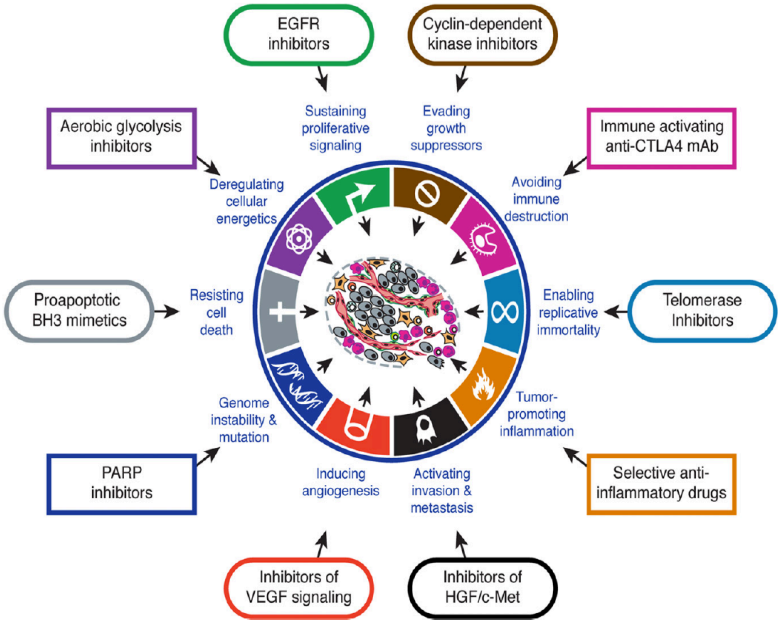
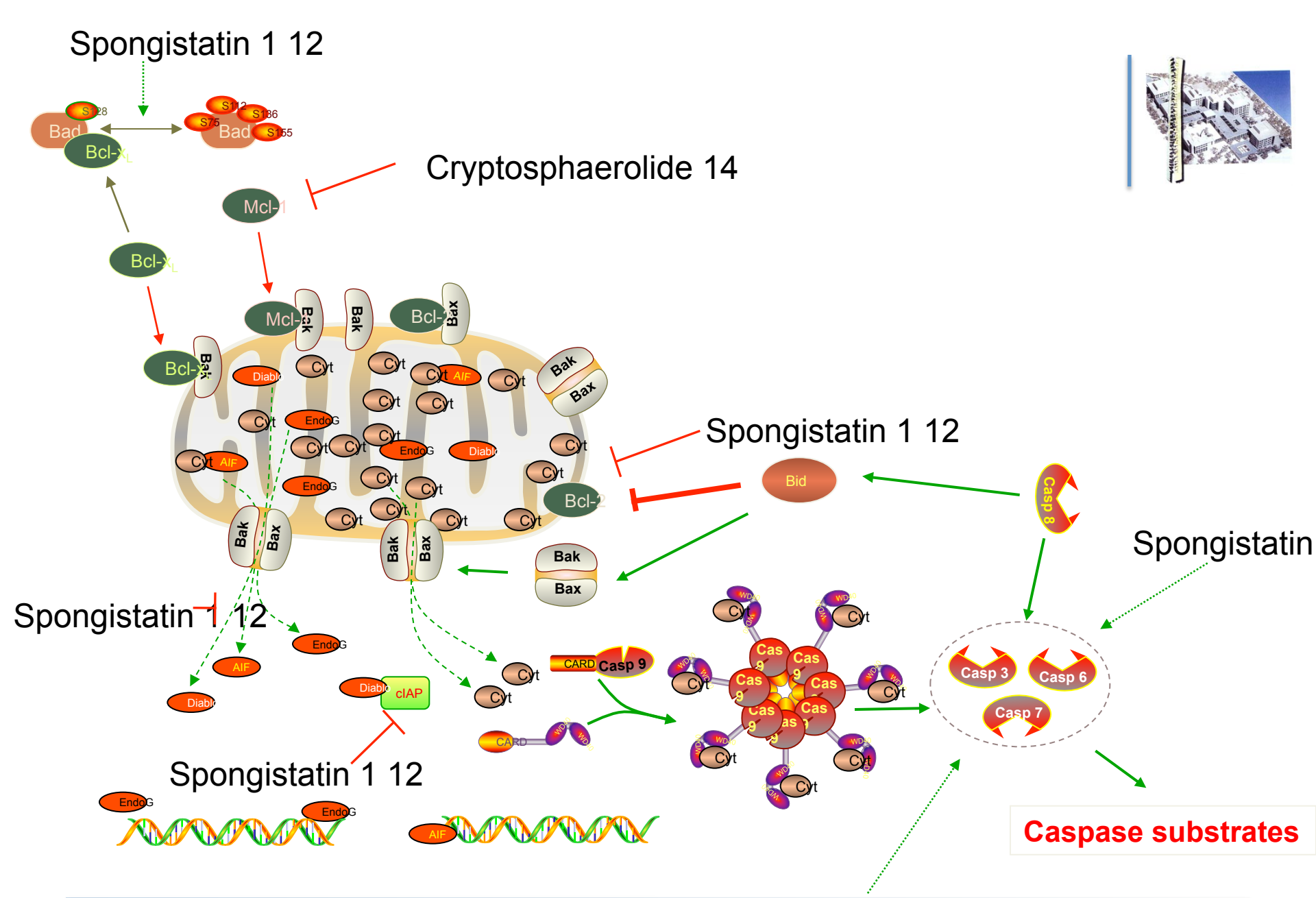


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

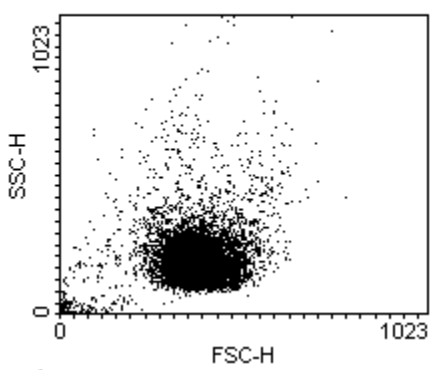
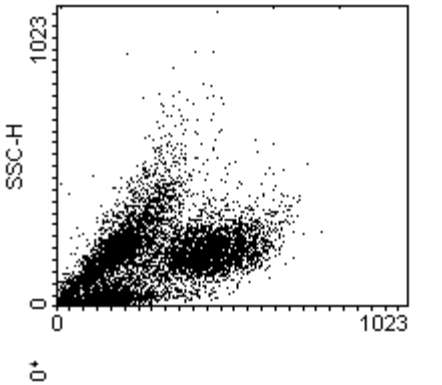
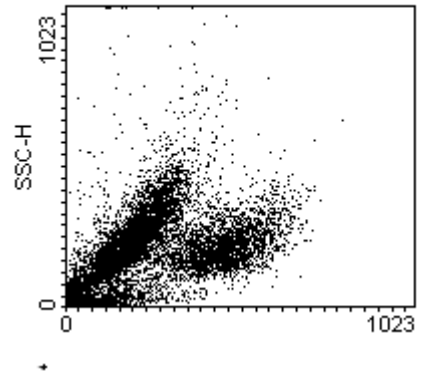
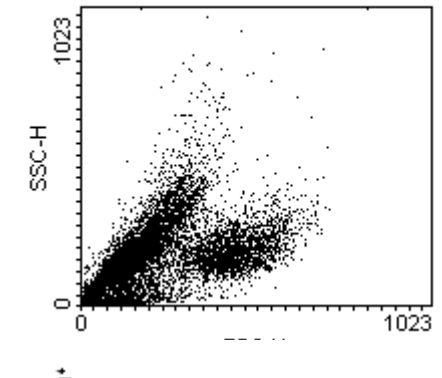
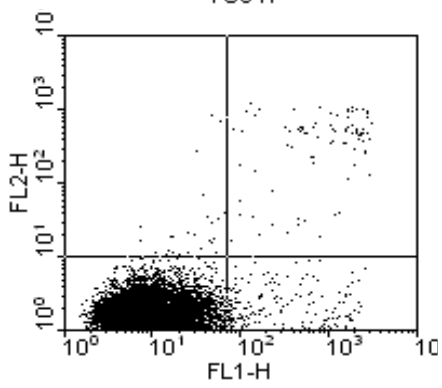
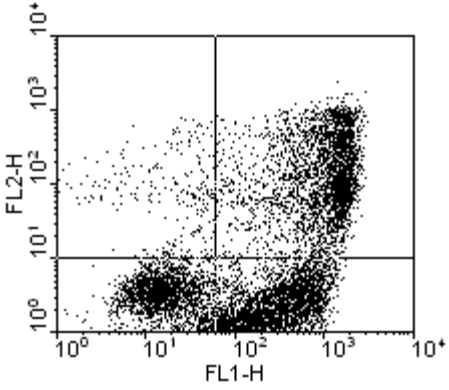
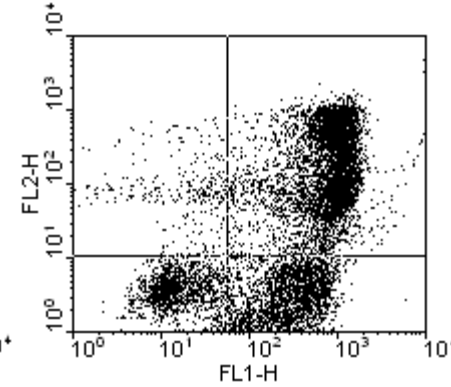
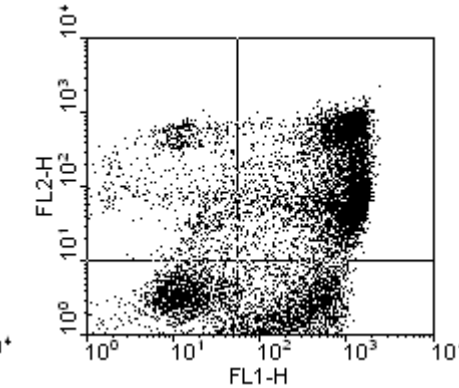


N-(4, 5-dibromo-pyrrole-2-carbonyl)-L-amino

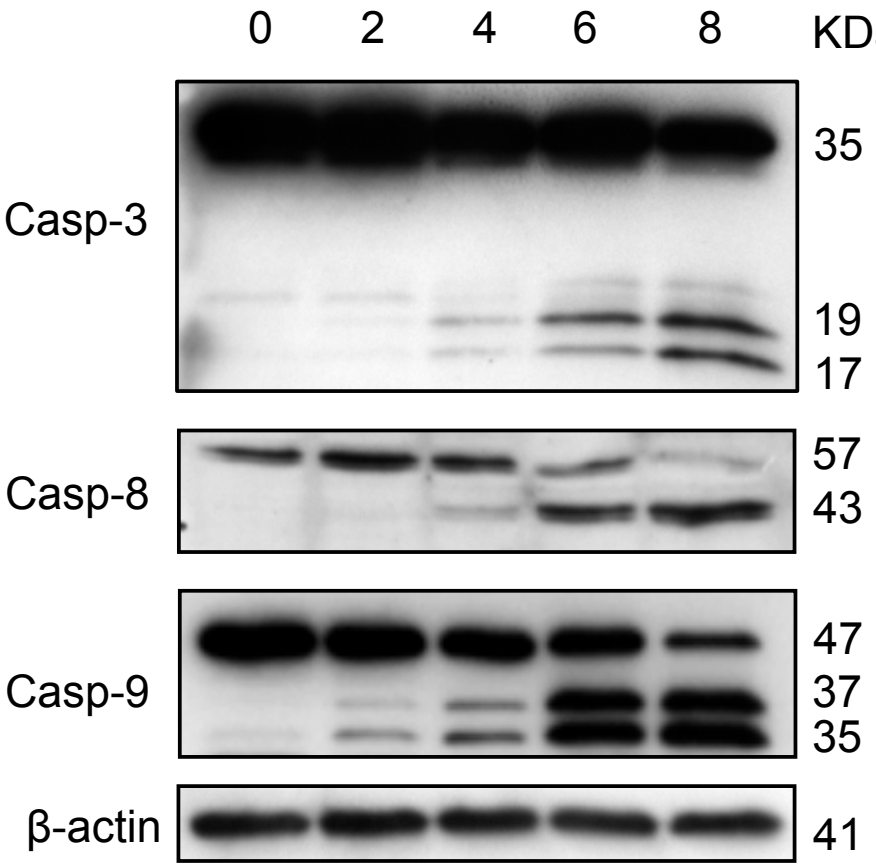
Fig 3B

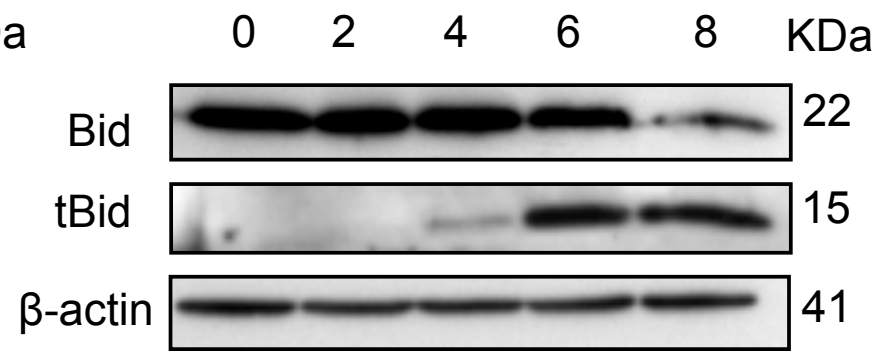
Induction of apoptosis

Heteronemin (8h of treatment) 0.1% FCS

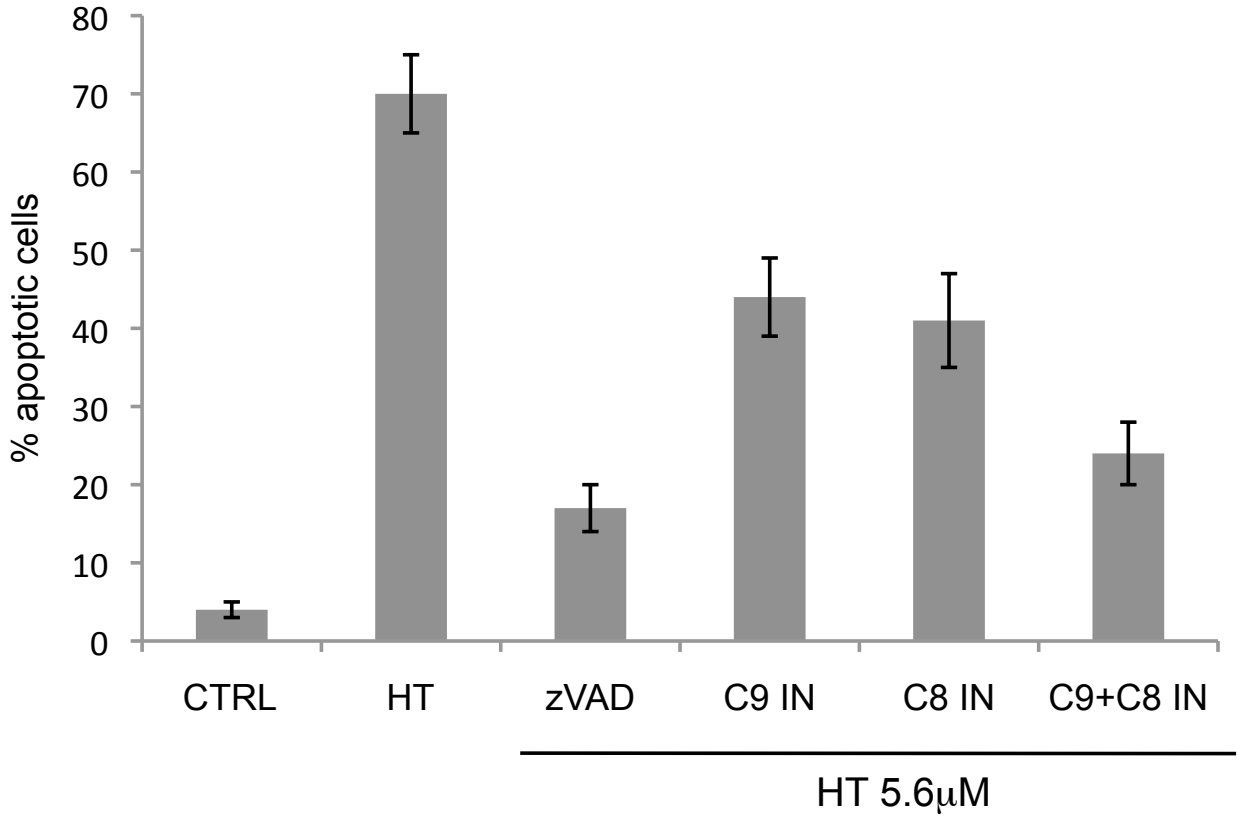
CTRL	4.0 μM	4.8 μM	5.6 μM
			
			
95% viable cells 5% dead cells	26% still viable cells 42% early apoptotic cells 29% late apoptotic cells	15% still viable cells 28% early apoptotic cells 53% late apoptotic cells	17% still viable cells 23% early apoptotic cells 52% late apoptotic cells

Caspase activation



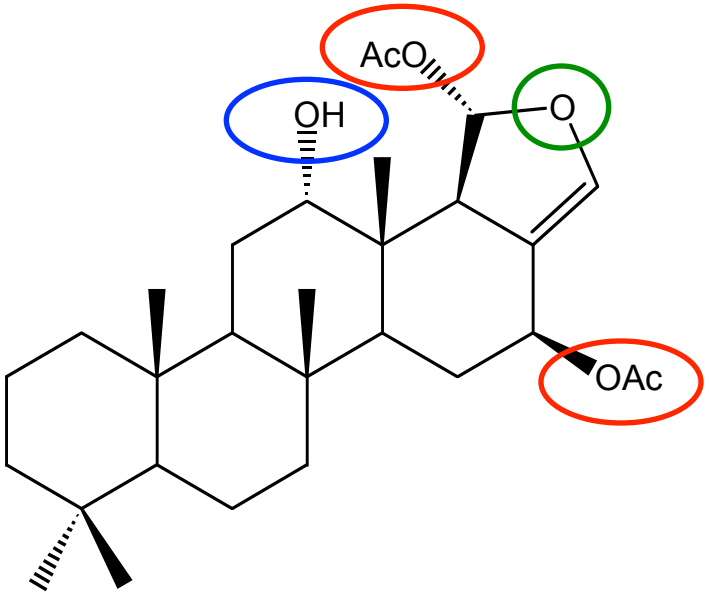


Caspase dependent cell death



Heteronemin

Promising chemical modifications:



Heteronemin

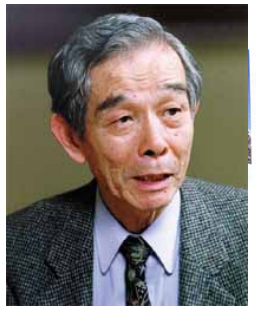
***) deprotection or protection**

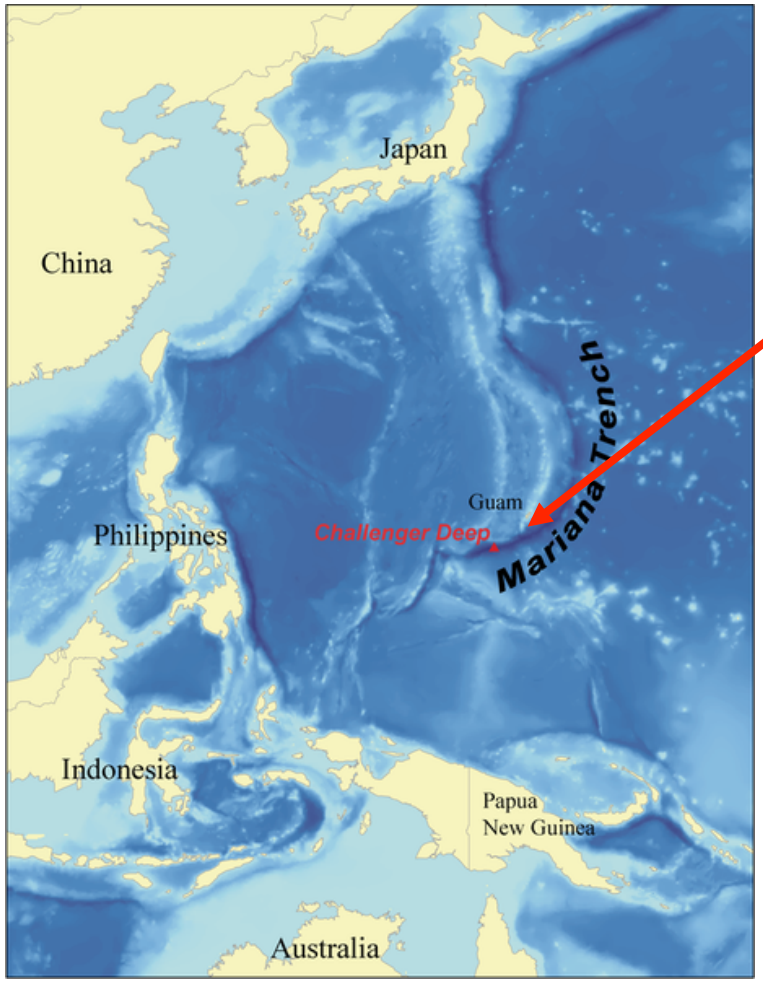
***) oxidation**

***) transformation into scaladiral (mild acid treatment)**

Explore unknown biological systems

The study of microorganisms from the Mariana trench



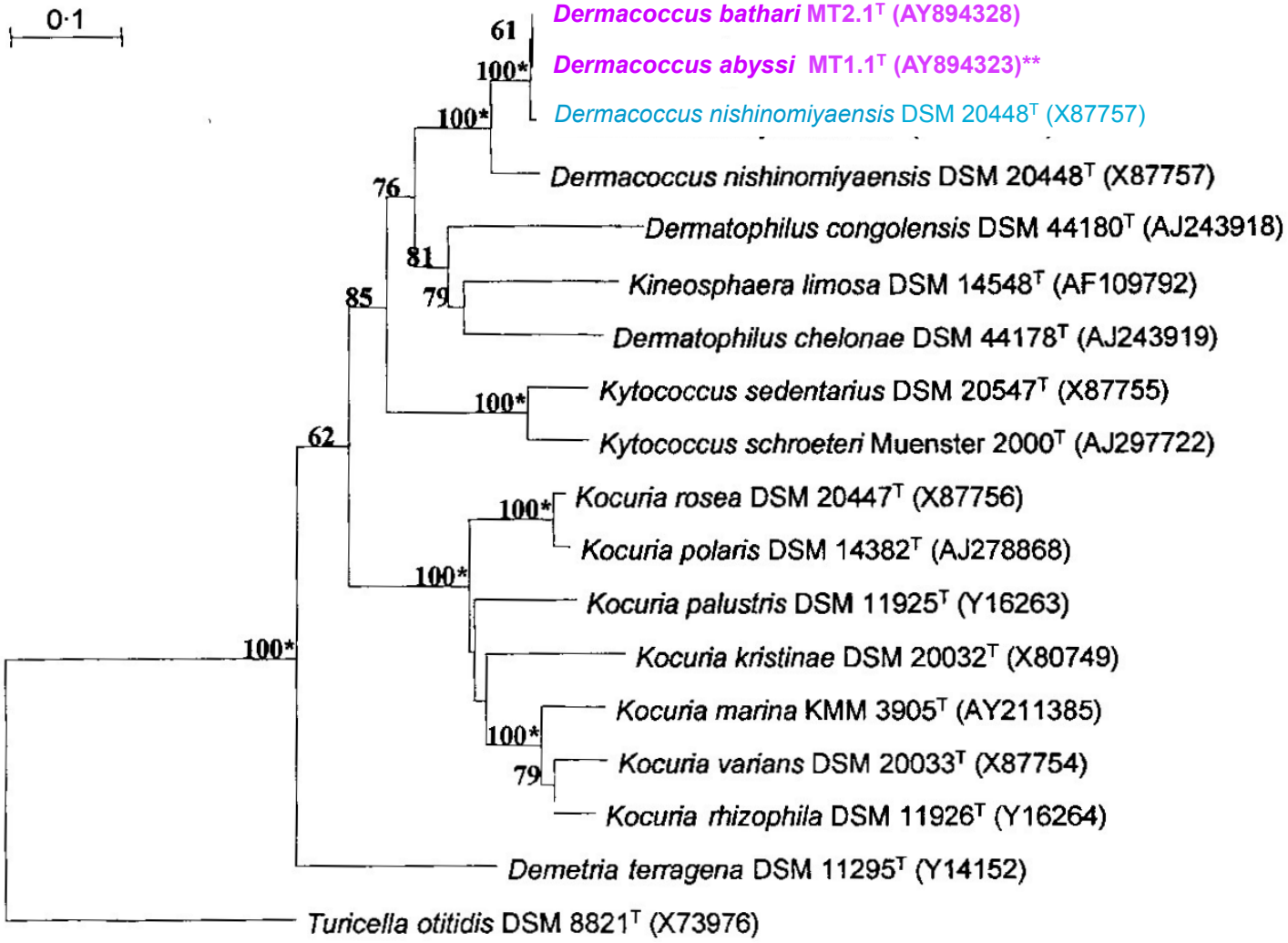


- **Challenger deep: -10911 m**
- **Access via JAMSTEC XBR (Prof Koki Horikoshi, Tokyo Institute of Technology)**

Kaiko – lost in 2003

in collaboration with Alan Bull, Mike Goodfellow, Koki Horikoshi

The genus *Dermacoccus*

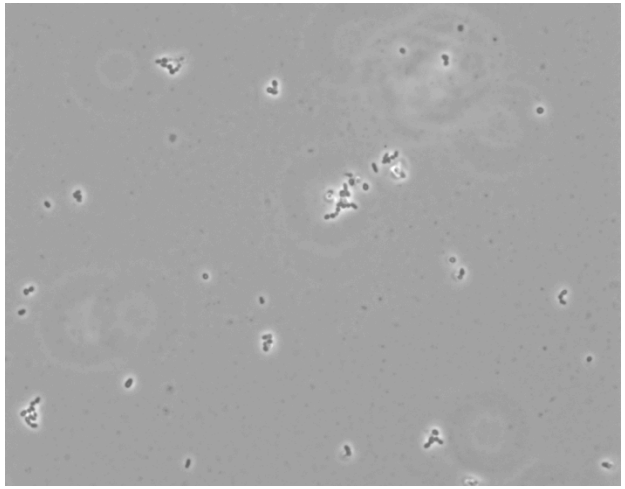


Pathom-aree *et al.* (2006) Int. J. System. Evol. Microbiol. 56: 2303

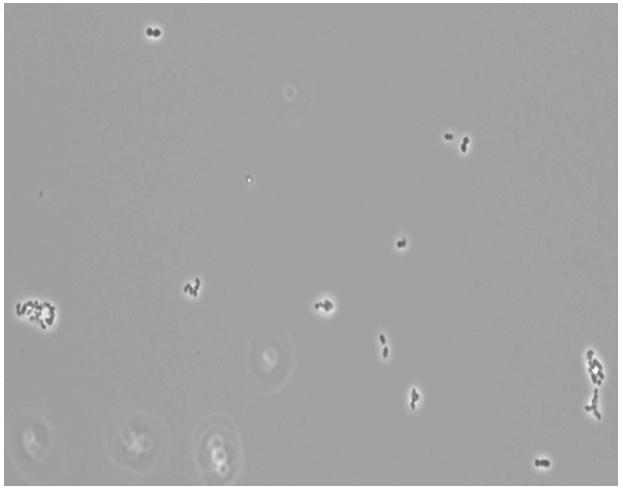
Dermacoccus abyssi, a piezotolerant bacterium

isolated from Mariana Trench sediment
at a depth of 10898 m.

Kingdom: Bacteria
Division: Actinobacteria
Order: Actinomycetales
Family: Dermacoccaceae
Genus: *Dermacoccus*
Species: *D. abyssi*



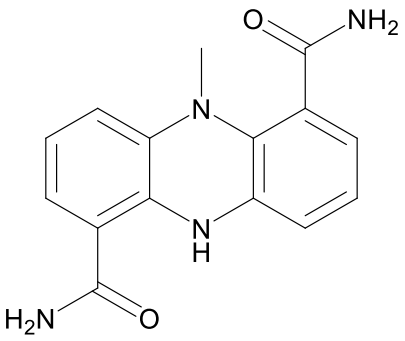
30°C, 0.1 MPa



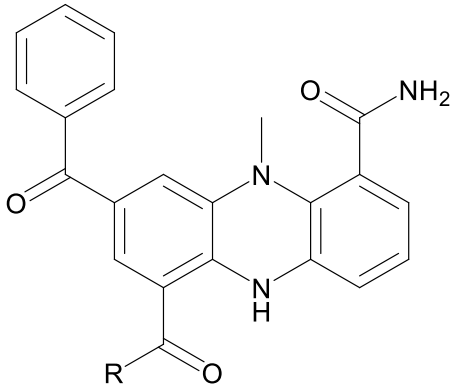
30°C, 40 MPa

Goodfellow, Bull et al. (2006) Int. J. Syst. Evol. Microbiol. 56: 1233

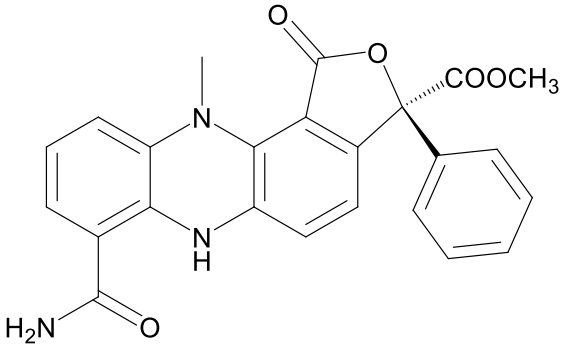
Dermacozines



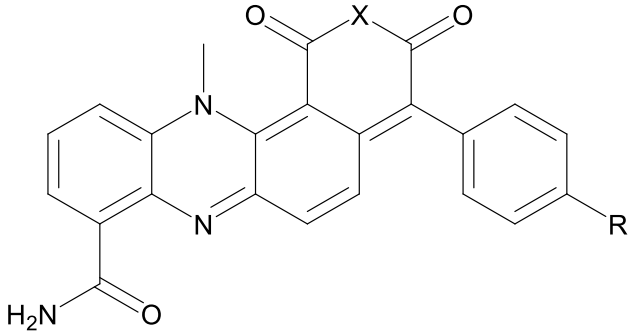
dermacozine A



dermacozine B ($R = NH_2$)
dermacozine C ($R = OH$)



dermacozine D



dermacozine E (X = NH, R = H)
dermacozine F (X = O, R = H)
dermacozine G (X = O, R = OH)

Abdel-Mageed et al. (2010) Org. Biomol. Chem. 8: 2352

PAPER www.rsc.org/obc | Organic & Biomolecular Chemistry

Dermacozines, a new phenazine family from deep-sea dermacocci isolated from a Mariana Trench sediment†

Wael M. Abdel-Mageed,[a,b] Bruce F. Milne,[c] Marcell Wagner,[d] Marc Schumacher,[e] Peter Sandor,[f] Wasu Pathom-aree,[g] Michael Goodfellow,[g] Alan T. Bull,[h] Koki Horikoshi,[i] Rainer Ebel,[a] Marc Diederich,[e] Hans-Peter Fiedler[d] and Marcel Jaspars*[a]

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DOI: 10.1039/c001445a

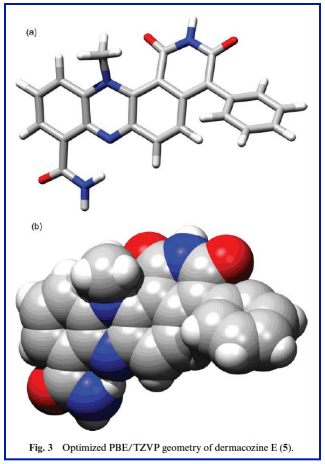


Fig. 3 Optimized PBE/TZVP geometry of dermacozine E (5).

Table 3. Cytotoxicity of Compounds 1−8 against K562 and PBMC Cells

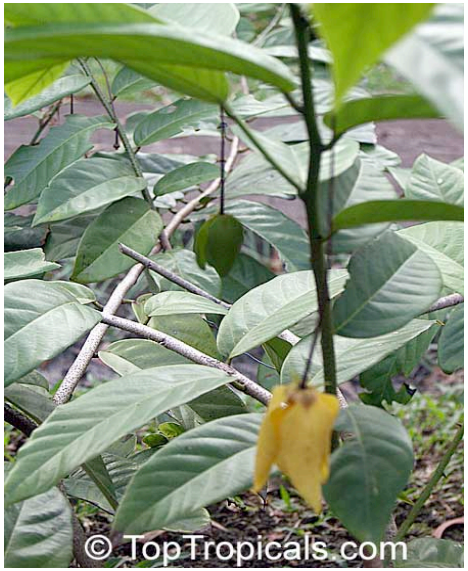
compound	IC_{50} values (μM)[a] K562	PBMC
1	15 ± 1	30 ± 10
2	8.5 ± 0.5	9 ± 1
3/4	4.5 ± 0.5	6.5 ± 1.5
5	0.6 ± 0.2	0.8 ± 0.4
6	2.65 ± 0.05	3.0 ± 0.5
7	2.3 ± 0.2	4.5 ± 0.7
8	12 ± 1	30 ± 10

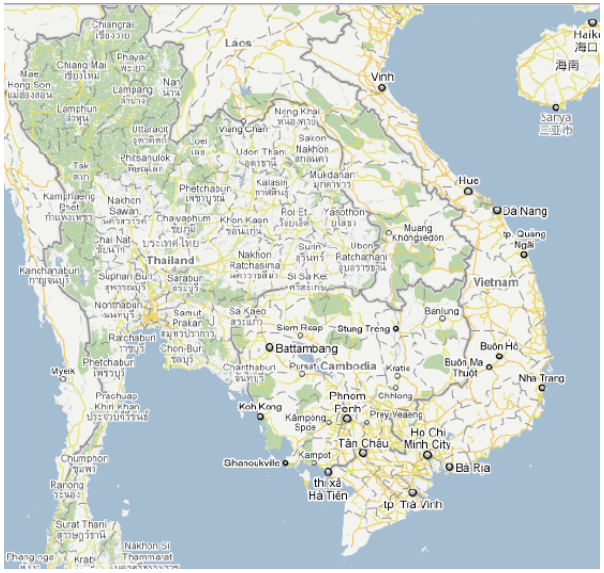
[a] The results are means ± standard deviation of three independent replicates.

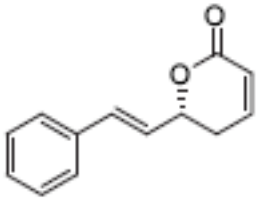
Table 4 Biological activity of the dermacozines

Compound	K562 Cytotoxicity IC_{50}/μM	DPPH radical scavenging activity IC_{50}/μM
Dermacozine A	140	77.5
Dermacozine B	220	38.0
Dermacozine C	180	8.4
Dermacozine D	100	106.9
Dermacozine E	145	—
Dermacozine F	9	—
Dermacozine G	7	—
Ascorbic acid		12.1

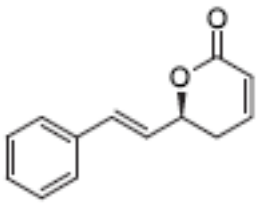
Bioassays: determination of cytotoxicity of novel compounds







(R)-Goniothalamin 1



(S)-Goniothalamin 1

chiral molecule

- **styryl lactone (5,6 dihydro-6-styryl-2-pyranone)**
- **synthesized by plants of the genus *Goniothalamus***
- **Bankok, Northern Thailand, Cambodia, Vietnam, Malaysia**
- **skin, bark, roots and leafs**

What is known about Goniothalamin

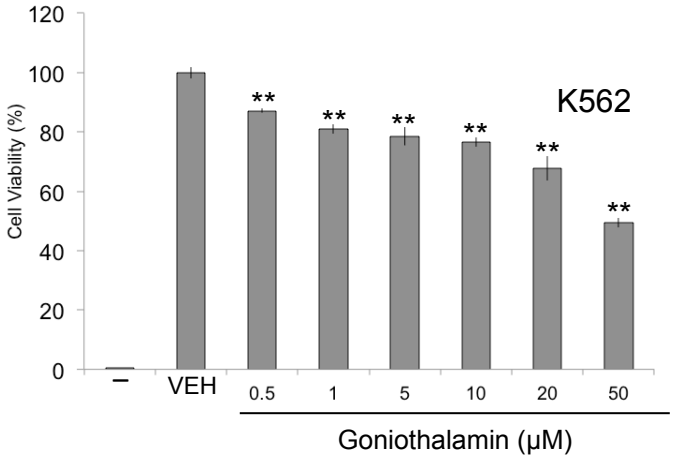
Caspases-3 and -7 are activated in goniothalamin-induced apoptosis in human Jurkat T-cells.
Inayat-Hussain SH, Osman AB, Din LB, Ali AM, Snowden RT, MacFarlane M, Cain K.
FEBS Lett. 1999 Aug 13;456(3):379-83.

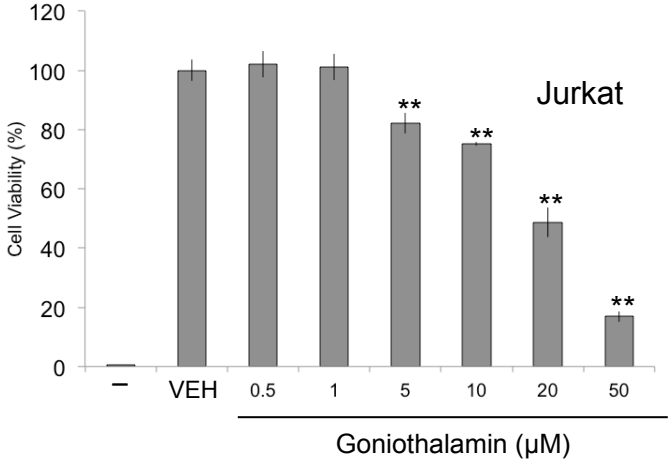
Loss of mitochondrial transmembrane potential and caspase-9 activation during apoptosis induced by the novel styryl-lactone goniothalamin in HL-60 leukemia cells.
Inayat-Hussain SH, Annuar BO, Din LB, Ali AM, Ross D.
Toxicol In Vitro. 2003 Aug;17(4):433-9.

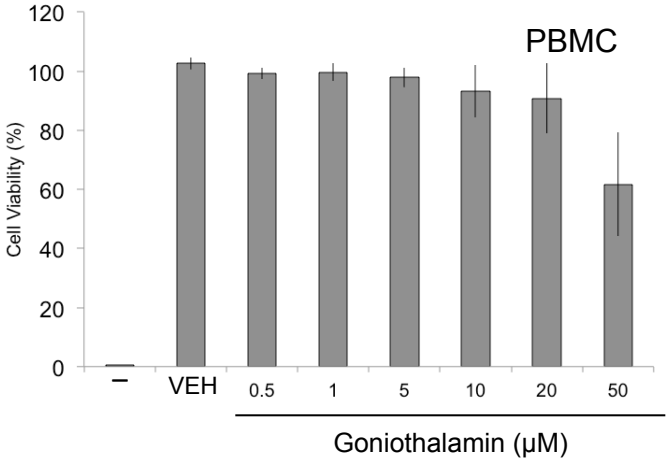
Cytotoxic activity of (S)-goniothalamin and analogues against human cancer cells.
Fátima A, Kohn LK, Carvalho JE, Pilli RA.
Bioorg Med Chem. 2006 Feb 1;14(3):622-31. Epub 2005 Oct 3.

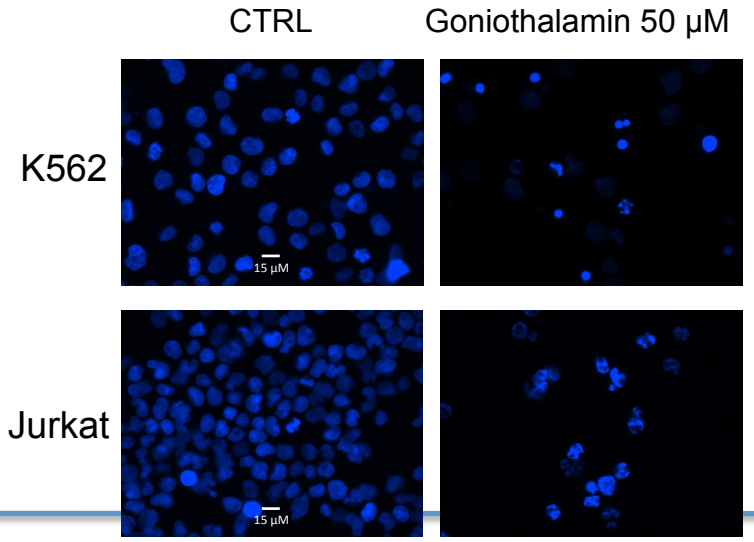
Goniothalamin-induced oxidative stress, DNA damage and apoptosis via caspase-2 independent and Bcl-2 independent pathways in Jurkat T-cells.
Inayat-Hussain SH, Chan KM, Rajab NF, Din LB, Chow SC, Kizilors A, Farzaneh F, Williams GT.
Toxicol Lett. 2010 Mar 1;193(1):108-14. Epub 2009 Dec 22.

Effect of Goniothalamin on cell viability



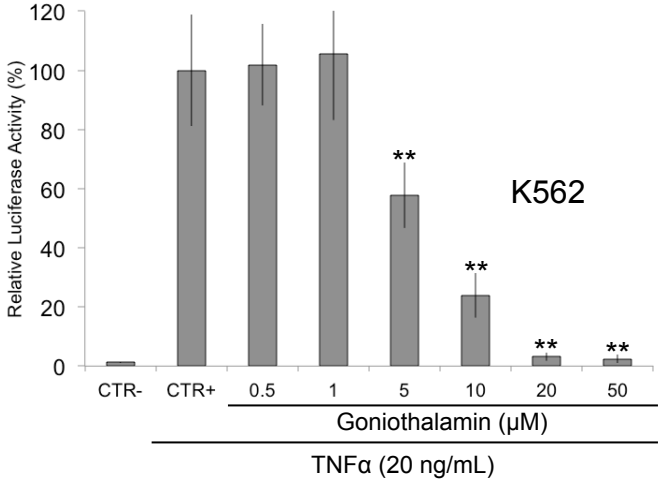


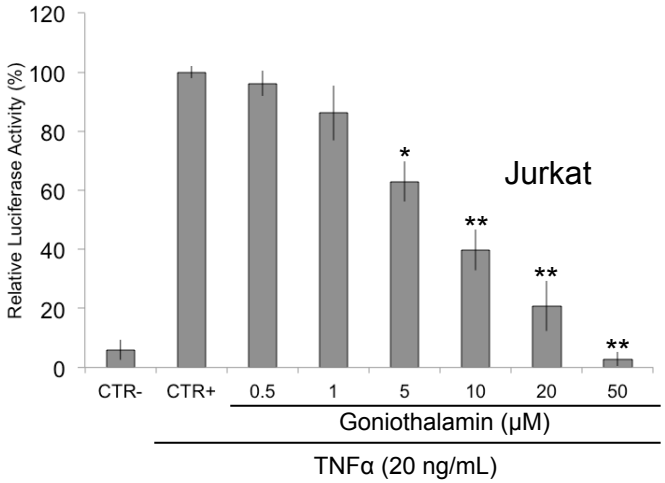




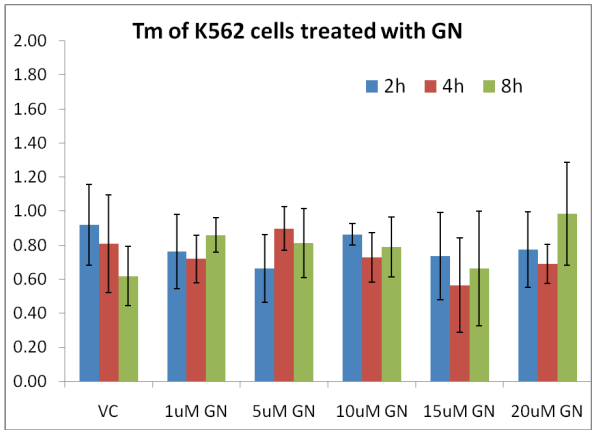
Effect on the NF-kB cell signaling pathway

A.

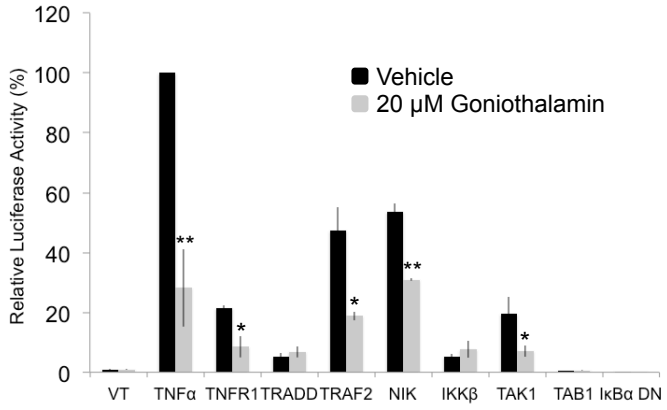




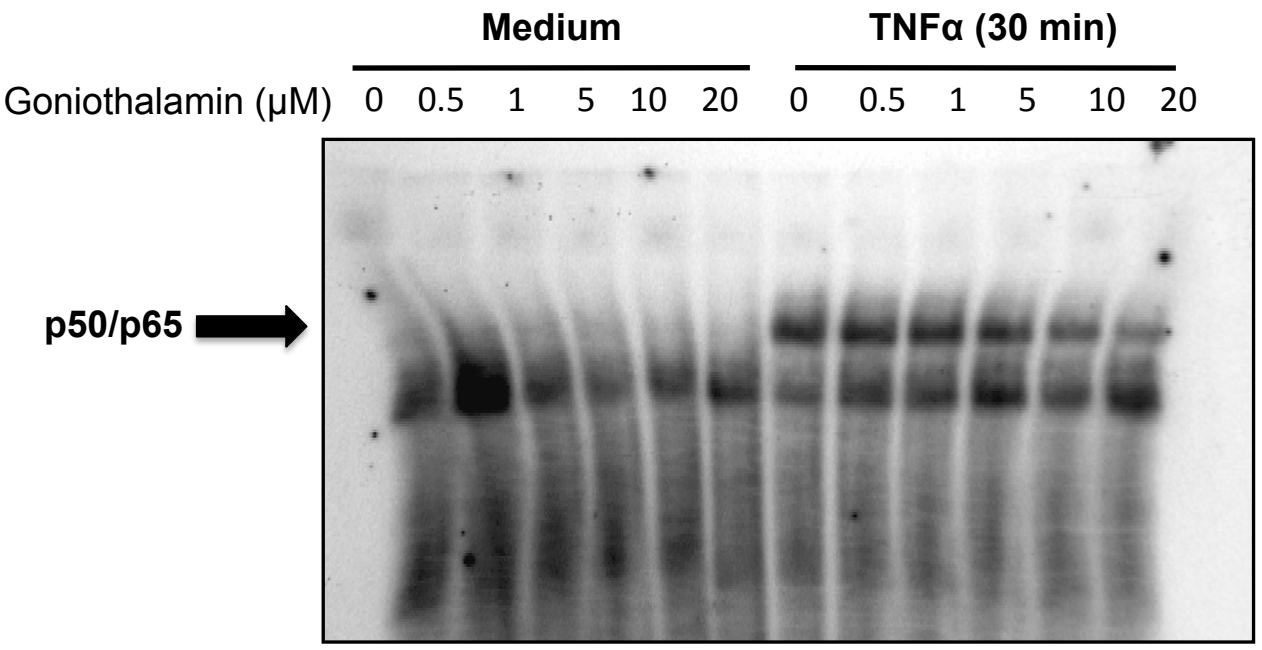
B.



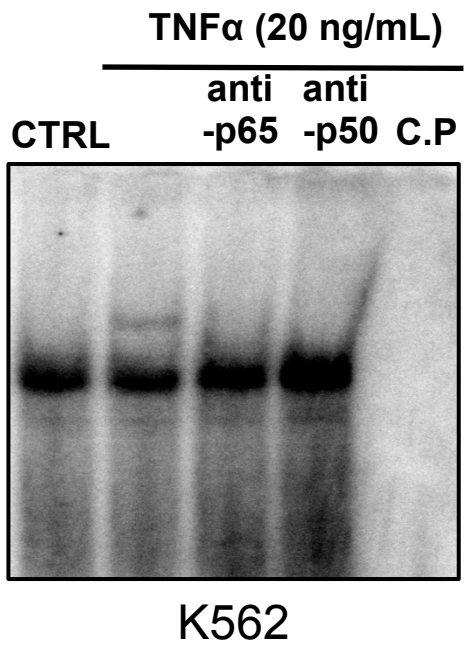
C.



Effect on the NF-kB cell signaling pathway



A.



B.

Cytoplasm

Medium

Goniothalamin (20 μM)

TNFα (min) 0 1 2 5 10 15 30 60 | 0 1 2 5 10 15 30 60

pIκBα ubiquitin.

pIκBα

IκBα

p50

p65

α-tubulin

Lamin B

kDa

40

39

50

65

60

65

Nucleus

p50

p65

α-tubulin

Lamin B

50

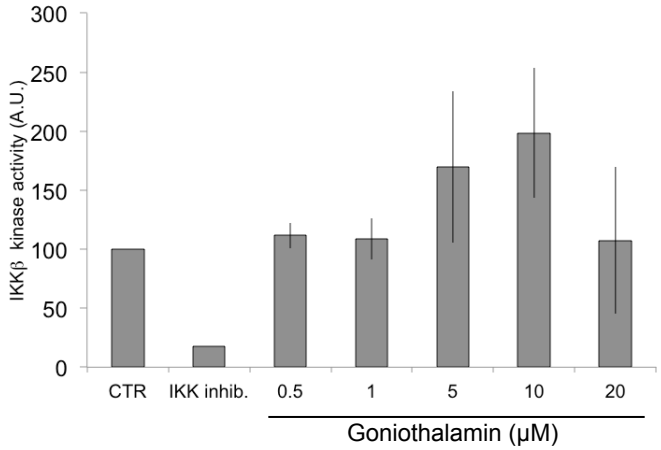
65

60

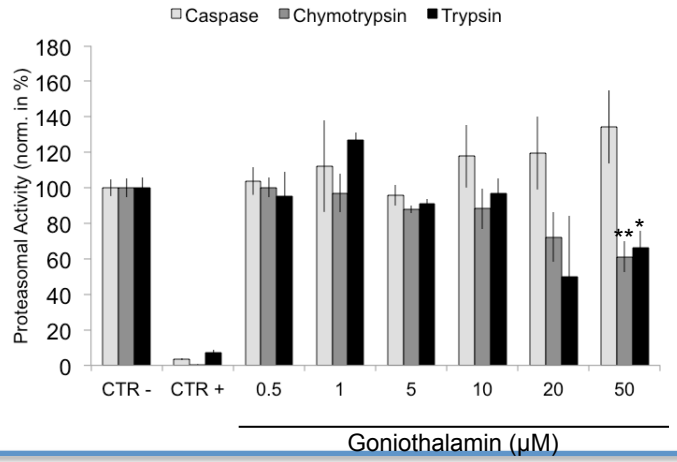
65

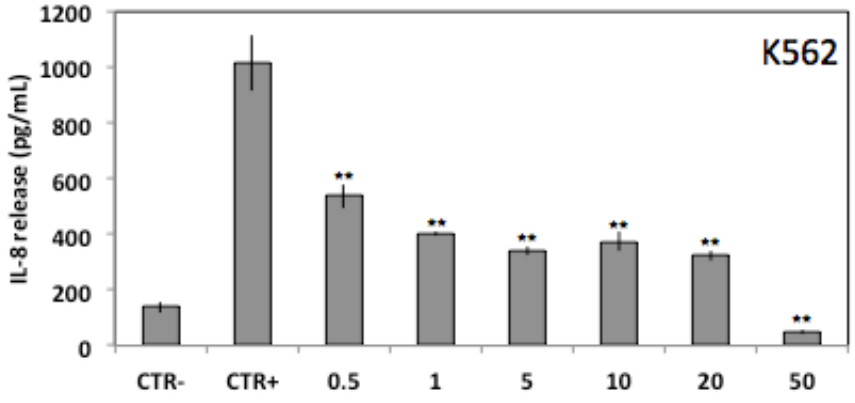
Effect on the NF-kB cell signaling pathway

A.



B.





Enabling characteristic: Genomic instability and mutation -> PARP-1 inhibitors

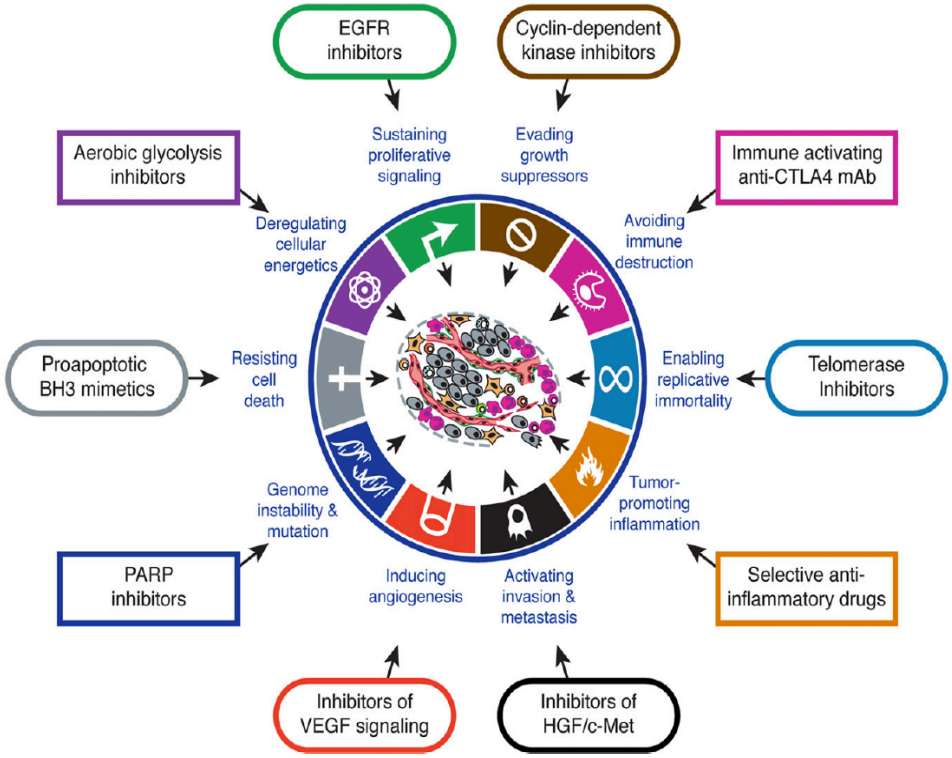


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

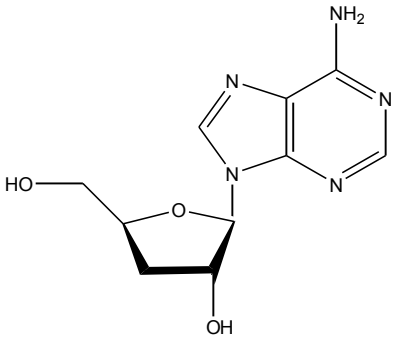
Genome instability

- Recently, discovery of the sensitivity of **homologous recombination-deficient** tumor cells to inhibition of **base excision repair** by targeting **poly(ADP-ribose) polymerase** (PARP)
- The use of PARP inhibitors to treat tumors with **defects in BRCA1/2** represents a novel interesting approach in addition to classical cancer therapy
- Promising activity of PARP inhibitors on high-grade serous ovarian carcinoma **missing germline BRCA1/2** mutations.
- **PARP inhibitors not only for the treatment of hereditary cancers but also for specific patient subclasses with common sporadic cancers**

From Orlikova and Diederich, submitted, 2011

PARP-1 inhibitors

- Many **synthetic molecules** with PARP inhibition potential
- One natural compound has been identified as a PARP inhibitor: **Cordycepin or 3'-deoxyadenosine** from the fungus *Cordyceps*



103
Cordycepin

- **anti-inflammatory potential**
- **inhibits PARP-1 activity in cell-free systems**
- **cordycepin (X) prevented H2O2-induced PARP activation in A549 lung cancer cells**
- **enhanced cell death of BRCA1-deficient MCF-7 breast cancer cells**
- **anti-inflammatory features favor its use in combinatory treatment in order to decrease inflammation processes activated by traditional chemotherapeutic agents.**

Cordyceps ophioglossoides

From review: Orlikova and Diederich, submitted, 2011

Hallmark: Cell cycle
-> evading growth suppressors

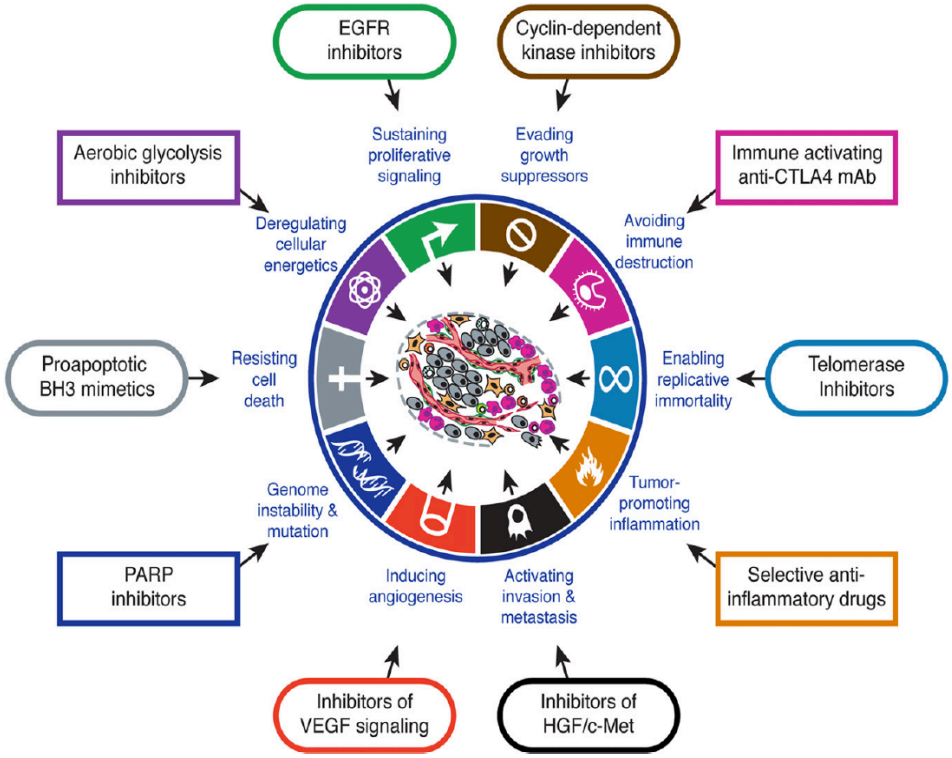
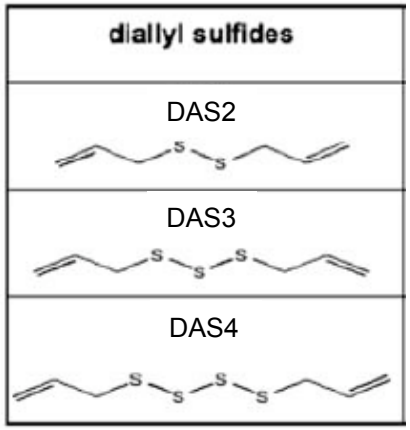


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

From review: Orlikova and Diederich, submitted, 2011

Garlic and cancer

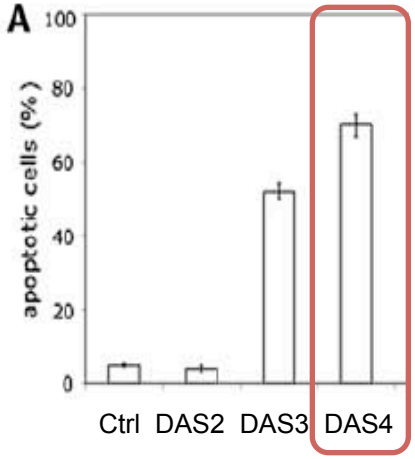
Epidemiological studies on garlic:
garlic consumption ⇕ ⟷ risk of cancer ⇓



- Preventive and therapeutic effects
- Not restricted to a special cancer type:
- Mainly against cancers of the gastrointestinal tract but also of peripheral organs (prostate, breast)

Most important biologically active compound class = organic sulfur compounds (OSCs):

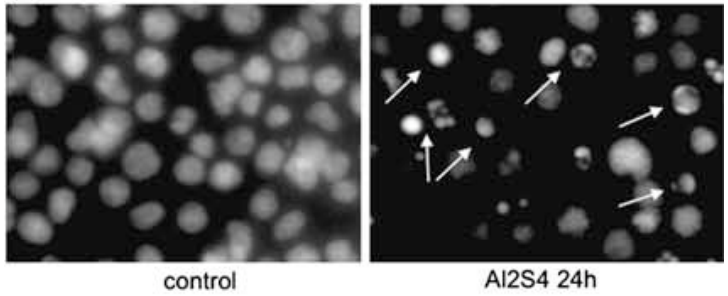
- selective anticancer/chemopreventive activity
- **Diallylpolysulfides:** direct correlation between number of sulfur atoms and anti-cancer potential



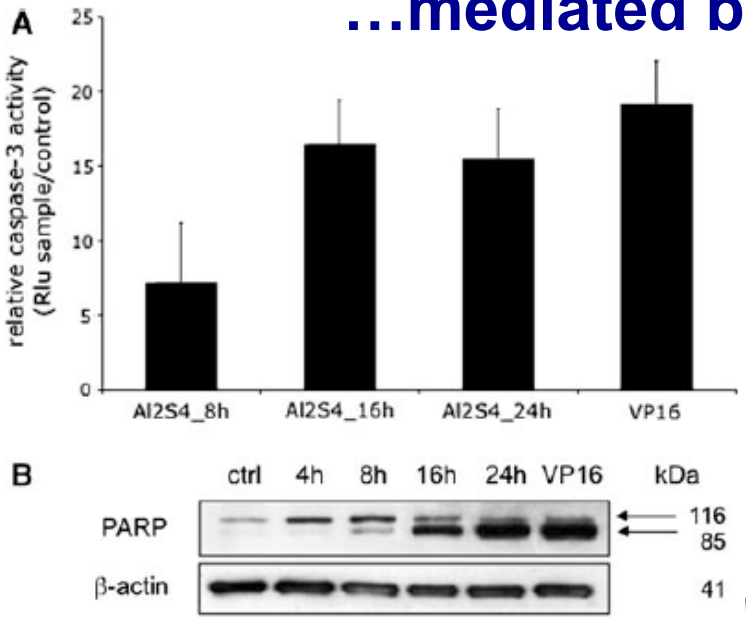
Cerella et al, Apoptosis, 2009

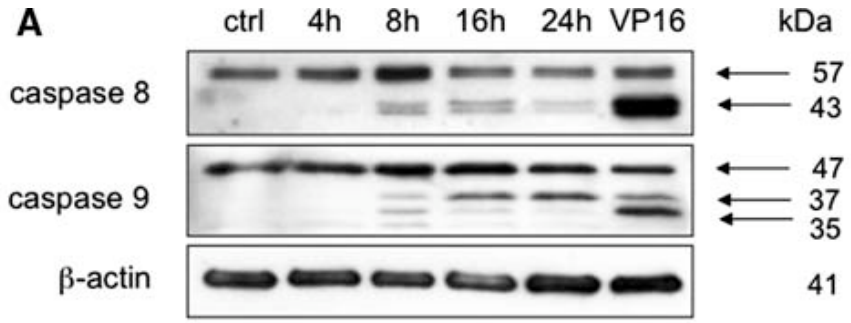
DAS4 induces apoptosis …

(in collaboration with Claus Jacob, Saarbrücken)



…mediated by activation of caspases



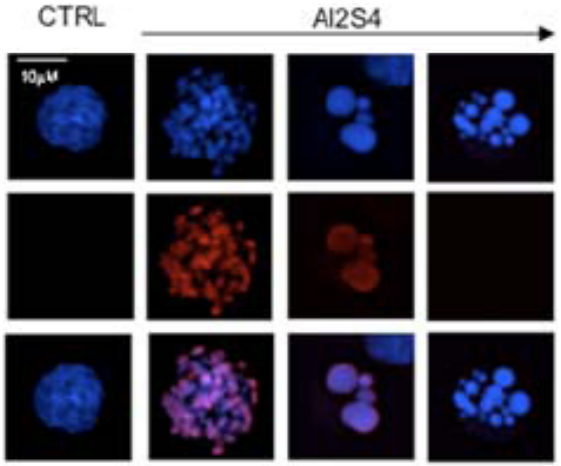


Cerella et al, Apoptosis, 2009

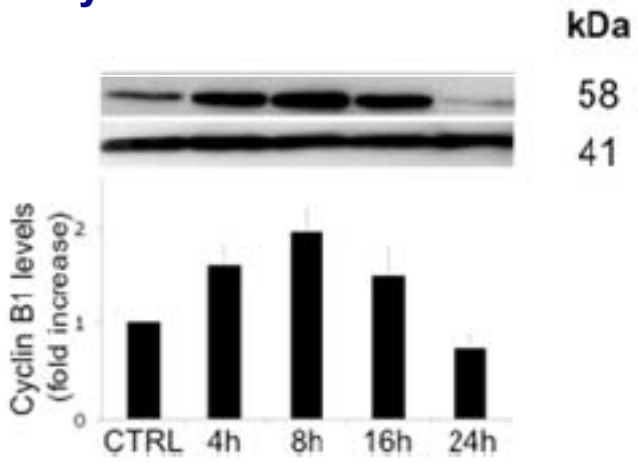
DAS4 arrests leukemia cells in early steps of mitosis

Accumulation of mitotic markers:

phosphorylated histone H3 (H3P)



Cyclin B1 accumulation



Cerella et al, Apoptosis, 2009

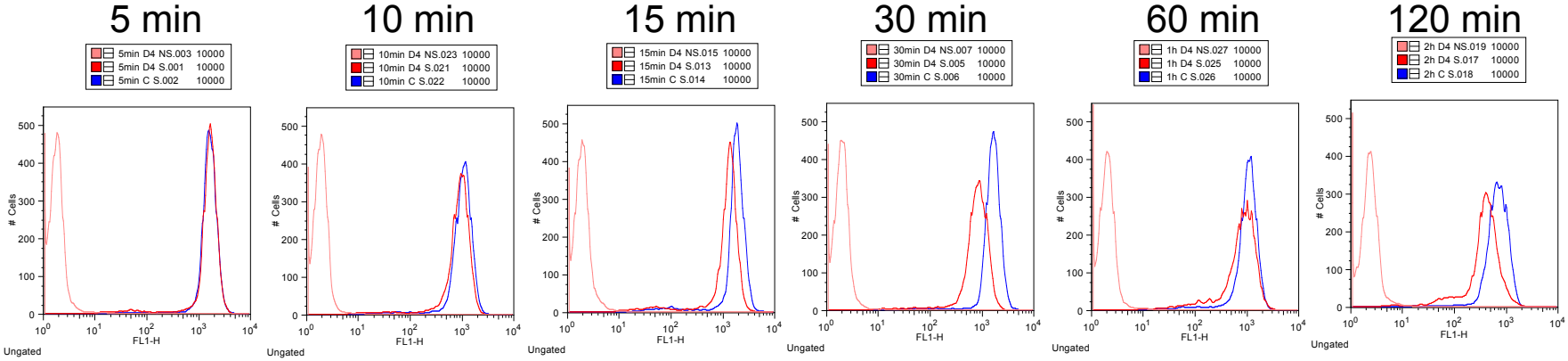
Are cell cycle arrest and apoptosis really induced by ROS?

What is the cellular target of DAS4?
Does it act *via* tubulin disorganisation?

What is the link between mitotic arrest and apoptosis?

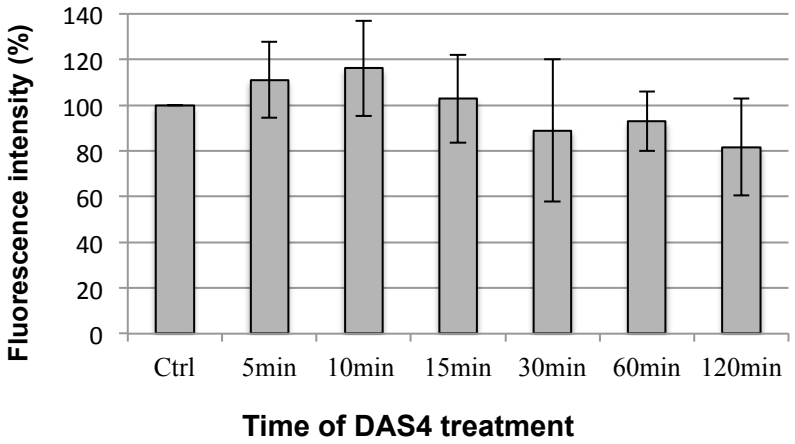
Kelkel, Cerella, Diederich et coll, 2011, manuscript in preparation

ROS staining



ROS accumulation after 5 + 10 min of DAS4 treatment is not significant

→ Is ROS really responsible for the induction of mitotic arrest and apoptosis?

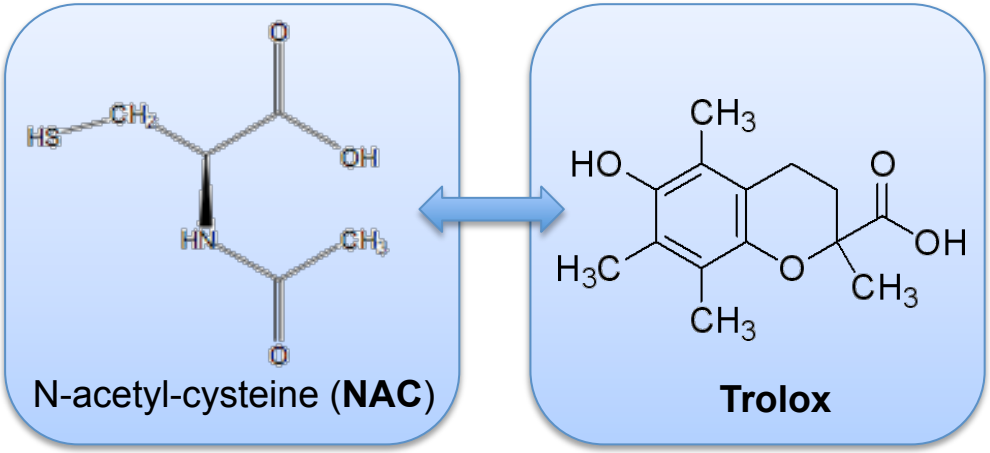


N=4

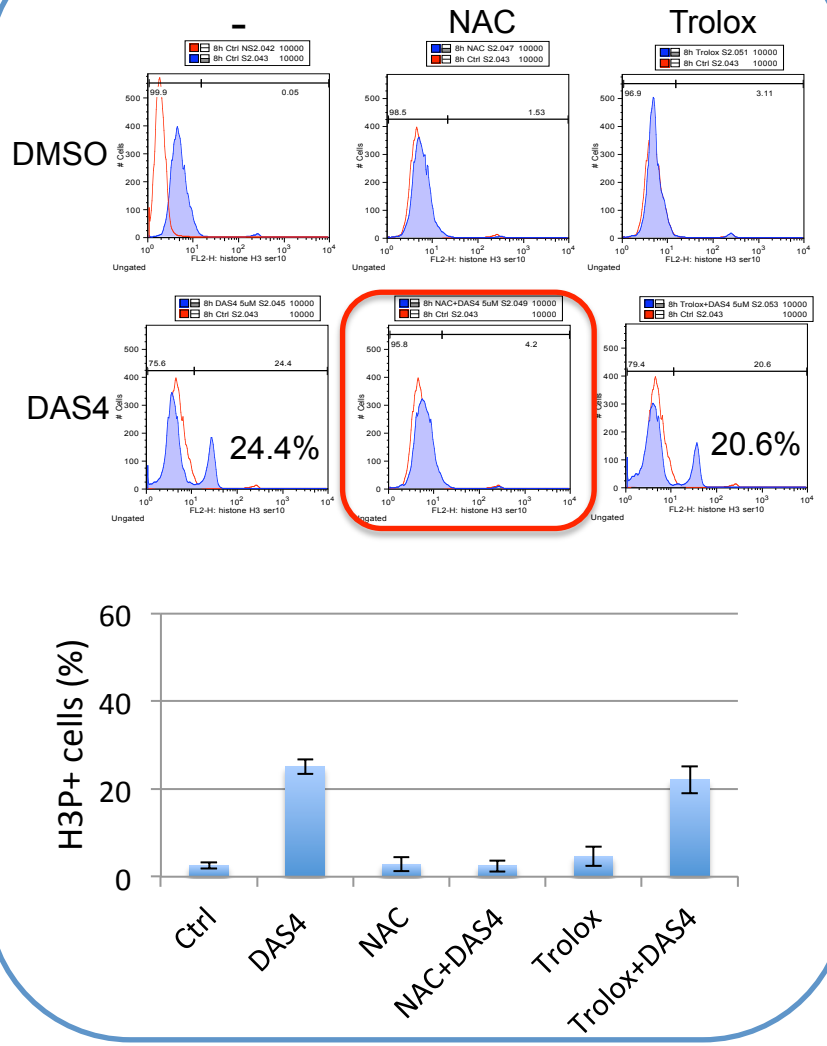
Kelkel, Cerella, Diederich et coll, 2011, manuscript in preparation

Differential effects of ROS scavengers

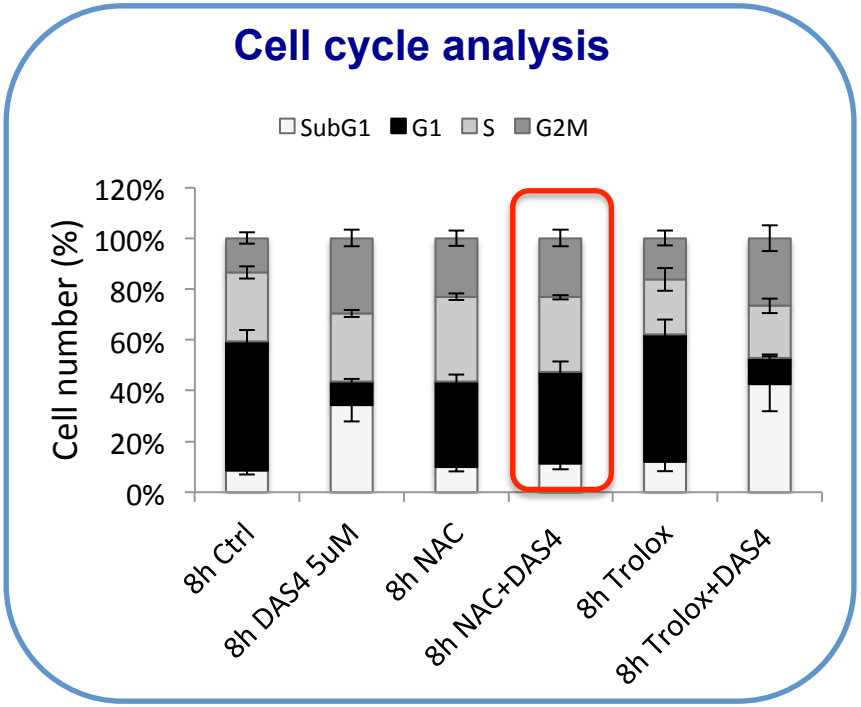
Treatment: 8h DAS4 (5µM)



H3P quantification

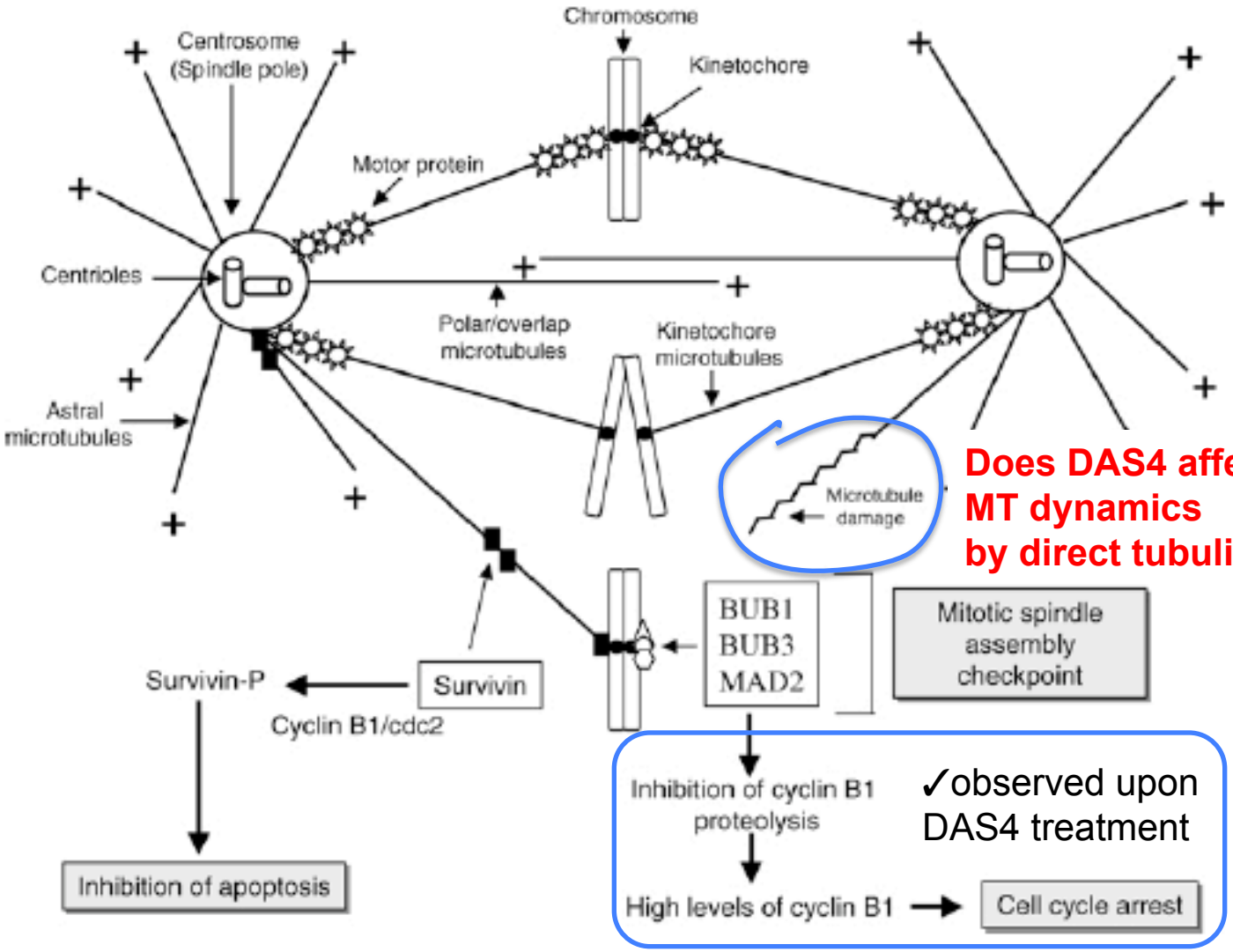


Cell cycle analysis



Kelkel, Cerella, Diederich et coll, 2011, manuscript in preparation

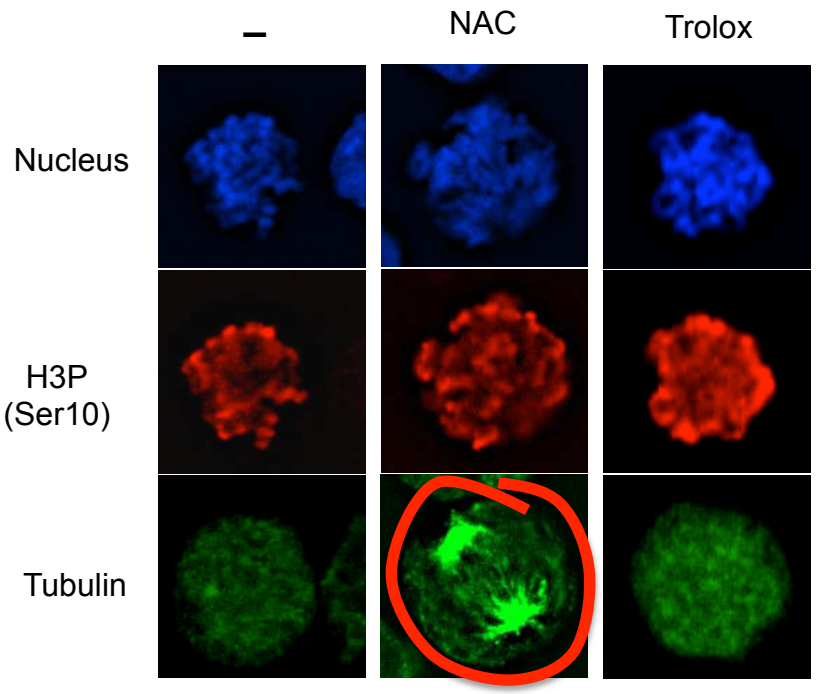
Mitotic spindle assembly checkpoint



Does DAS4 affect MT dynamics by direct tubulin binding?

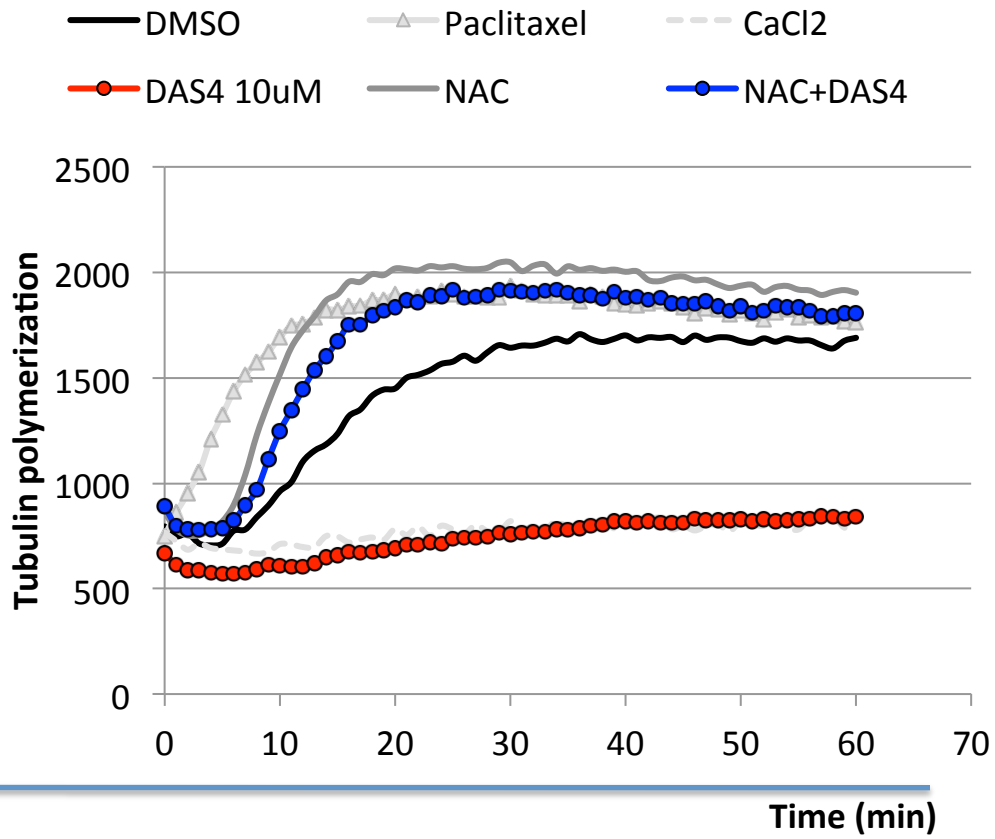
✓observed upon DAS4 treatment

8h treatment with 5 µM DAS4



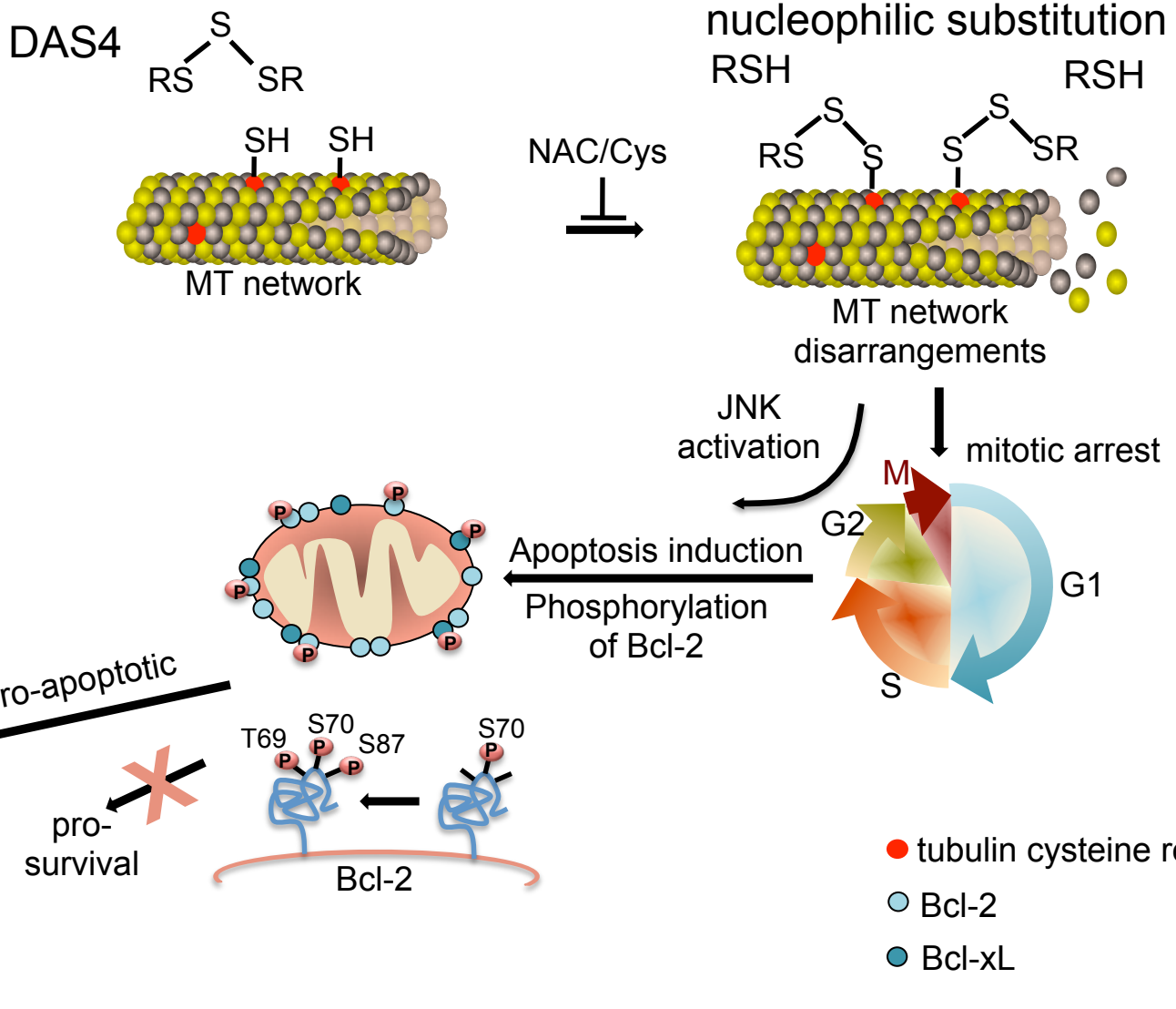
NAC counteracts DAS4-induced tubulin depolymerization

Only NAC restores normal microtubule structures in DAS4-treated cells



Kelkel, Cerella, Diederich et coll, 2011, manuscript in preparation

Modell of the action of DAS4



Kelkel, Cerella, Diederich et coll, 2011, manuscript in preparation

Hall mark: Activated proliferative signaling
-> Receptor tyrosine kinases

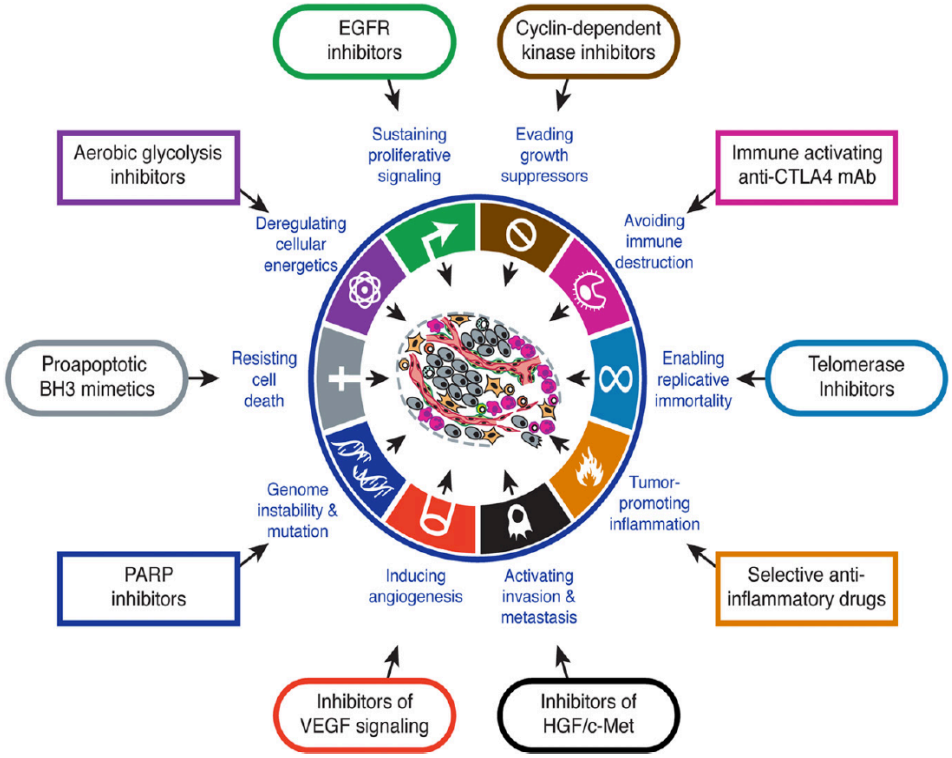


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

Epigallocathechin 3-gallate (EGCG) (1)

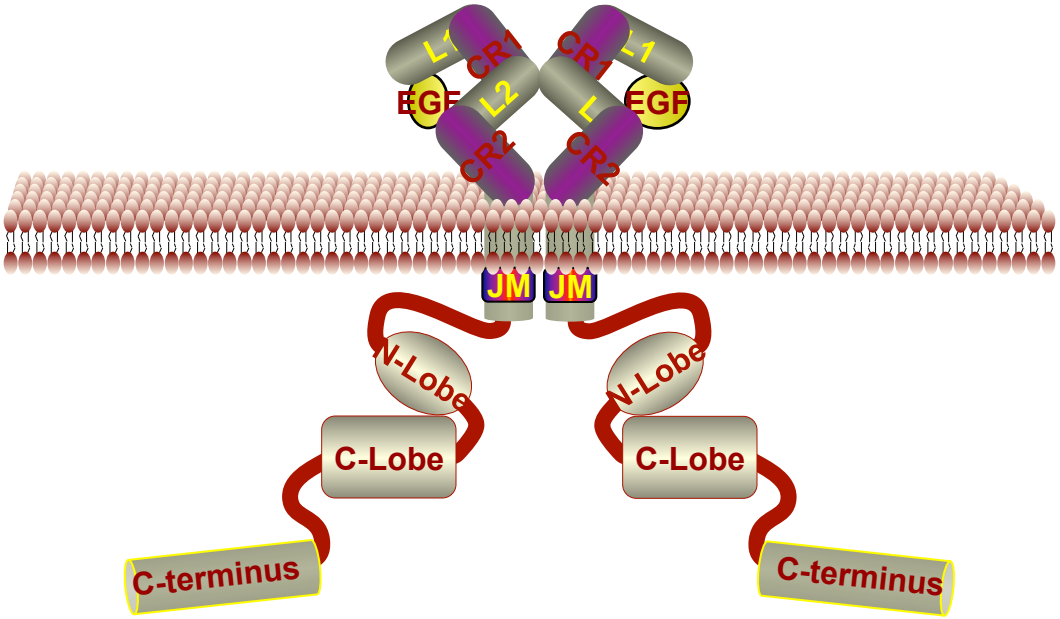
Quercetin (12)

Theaflavin digallate (2)

Tephrosin (11)

Curcumin (3)

Genistein (10)



Phloretin (9)

Capsaicin (4)

Marein (8)

Delphinidin (5)

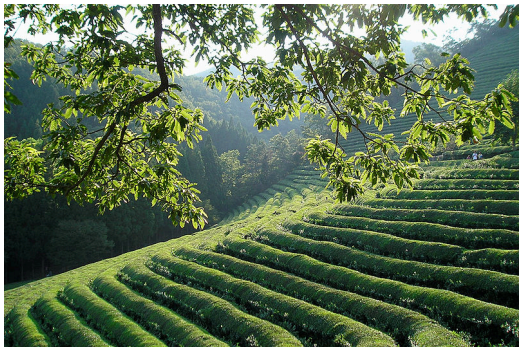
Butein (7)

N-coumaroyltyramine (6)

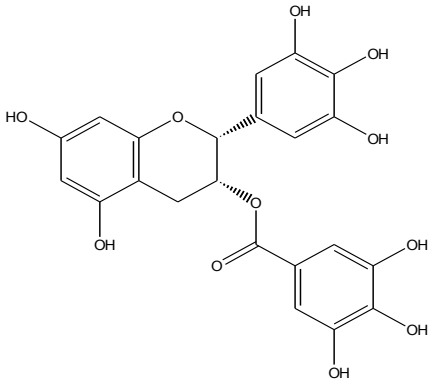
From review: Orlikova and Diederich, submitted, 2011

Inhibitors of RTKs

- Green tea polyphenol **epigallocatechin 3-gallate (EGCG)**, has been described as a potent inhibitor of critical RTKs, namely, EGFR, HER2, HER3 in human colon cancer
  - EGCG impaired phosphorylation of insulin like growth factor 1 receptor (IGF-1R), which is linked in some cases to resistance to treatment by EGFR inhibitors
  - EGFR degradation is due to serine 1046/1047 phosphorylation, mediated *via* EGCG-induced p38 MAPK activation
  - EGCG appears to be an interesting synergistic therapeutic supplement together with classical anticancer agents in order to overcome tumor-treatment resistance in various malignancies



Camelliaa sinensis

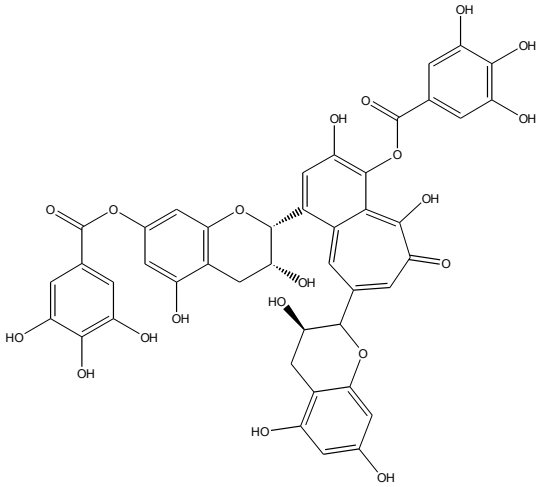


1 Epigallocatechin 3-gallate

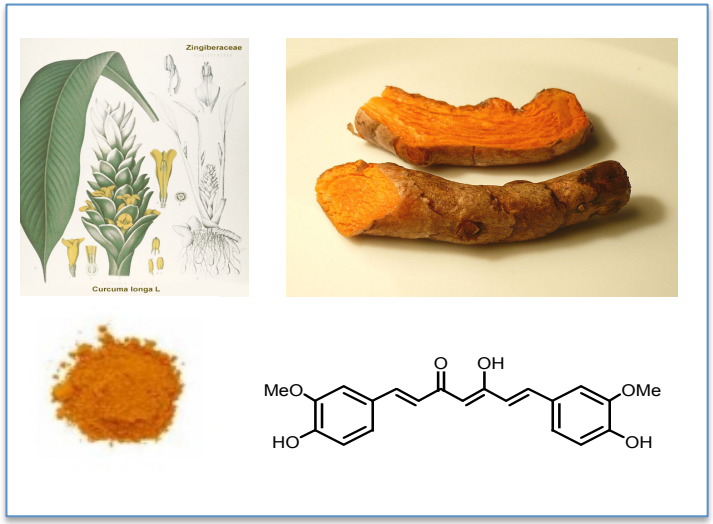
From review: Orlikova and Diederich, submitted, 2011

Inhibitors of RTKs

- Components from black tea such as **theaflavin digallate** induced EGFR endocytosis and consequent degradation



2 Theaflavin digallate



- **Curcumin** treatment potentiates gefitinib efficiency in resistant non-small lung cancer cells by downregulation of EGFR phosphorylation and induction of EGFR ubiquitination.

From review: Orlikova and Diederich, submitted, 2011

Hallmark: Increased migration and metastasis

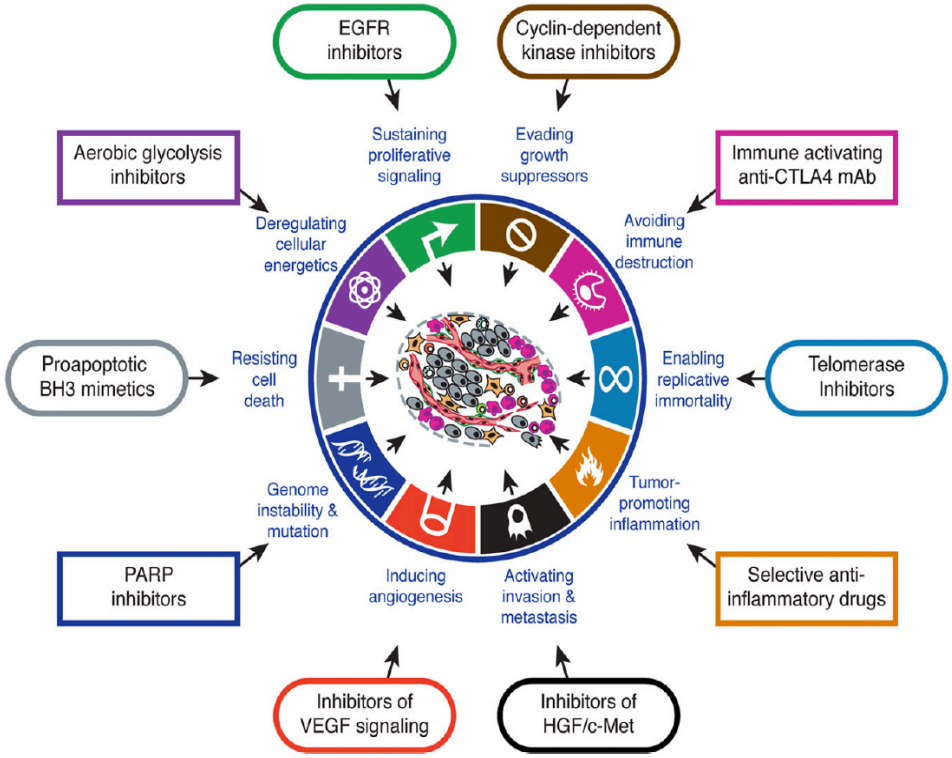
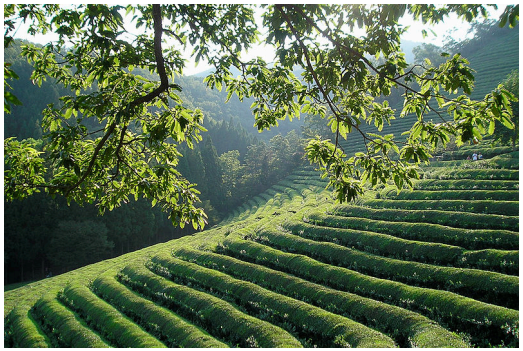


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

Inhibitors of invasion

- **Various polyphenols reduced activation of proteases, preferentially MMP-2 and MMP-9**
  - epigallocatechin-3-gallate from **green tea**
  - Resveratrol from **grapes**/red wine
  - Allyl isothiocyanate and its N-acetylcysteine conjugates from **cruciferous vegetables**



Camelliaa sinensis

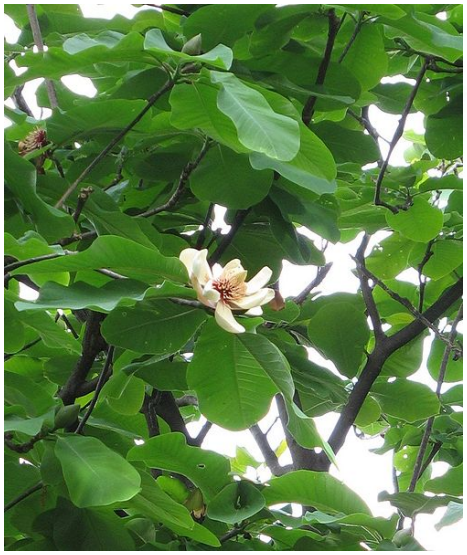
Vitis vinifera

Brassica oleracea viridis

From review: Orlikova and Diederich, submitted, 2011

Pothomorphe umbellata
4-nerolidylcatechol

Zingiber officinale
6-gingerol



Magnolia obovata
Obovatal

Euonymous alatus
Chlorogenic acid and caffeic acid

Glycyrrhiza uralensis
Licoricidin

Hypericum calycinum
Hyperforin

Inhibitors of invasion

Hallmark: Enabling replicative immortality -> Telomerase

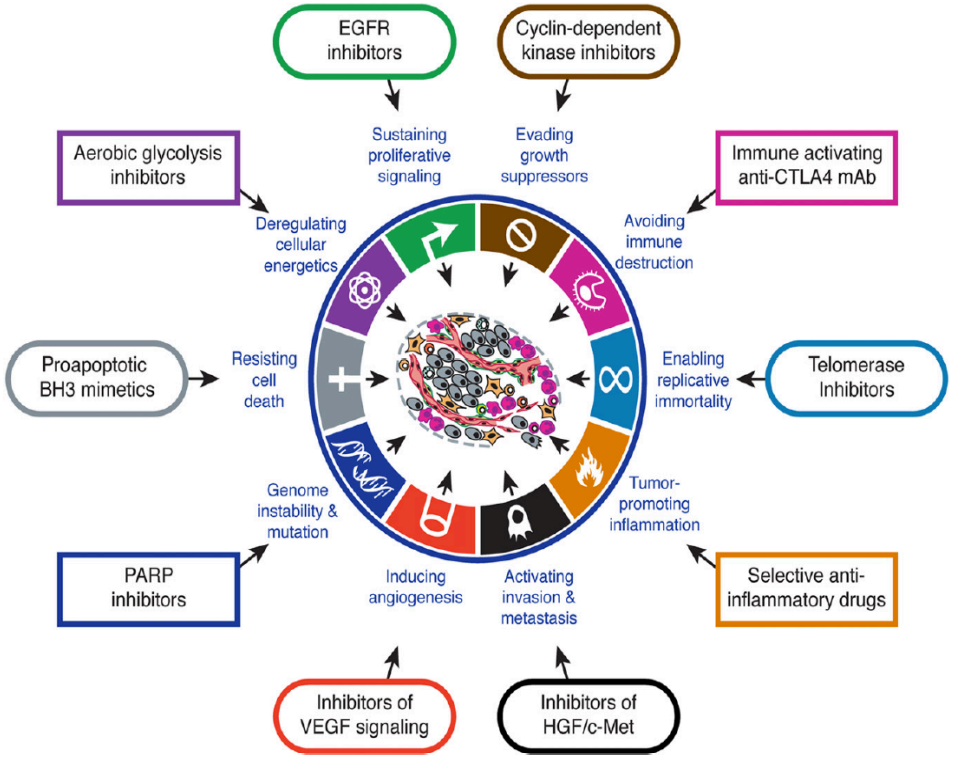
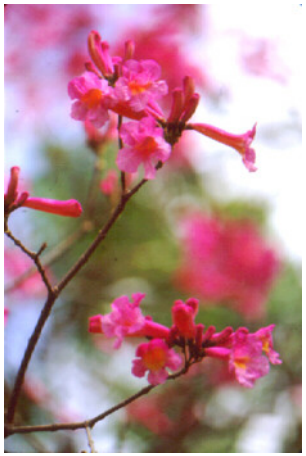


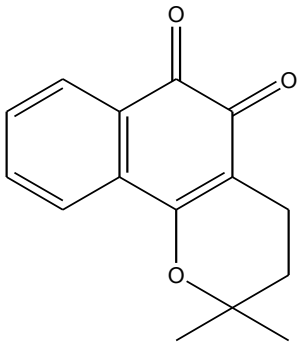
Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

Telomerase inhibitors

- Targeting of **core telomerase components**, namely, a functional **telomerase RNA (hTER)** and/ or the catalytic subunit - **telomerase reverse transcriptase (hTERT)**.
- This complex has been shown to be a target for **curcumin**-mediated dose and time-dependent repression of telomerase activity.
  - **Protection of hTERT nuclear translocation** in K-562 human leukemia cells upon curcumin treatment.
- **Telomerase RNA** appeared to be a target for β-lapachone, a quinone isolated from the bark of the Lapacho tree *Tabebuia.*
  -> Reduction of the telomerase RNA level together with a down-regulation of c-myc expression in the human lung carcinoma cell line A549.



Tabebuia impetiginosa

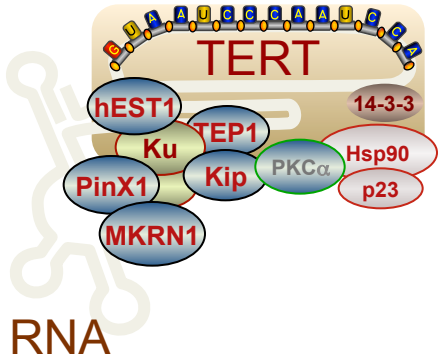


28 Beta-lapachone

From review: Orlikova and Diederich, submitted, 2011

Sulfoquinovosyl diacylglycerol (32)

Malouetine (34)



Wogonin (30)

β-Lapachone (28)

Silibinin (31)

Telomestatin (33)

Epigallocatechin -3-gallate (1)

Funtumine guanylhydrazone (35)

From review: Orlikova and Diederich, submitted, 2011

Hallmark: Increased angiogenesis

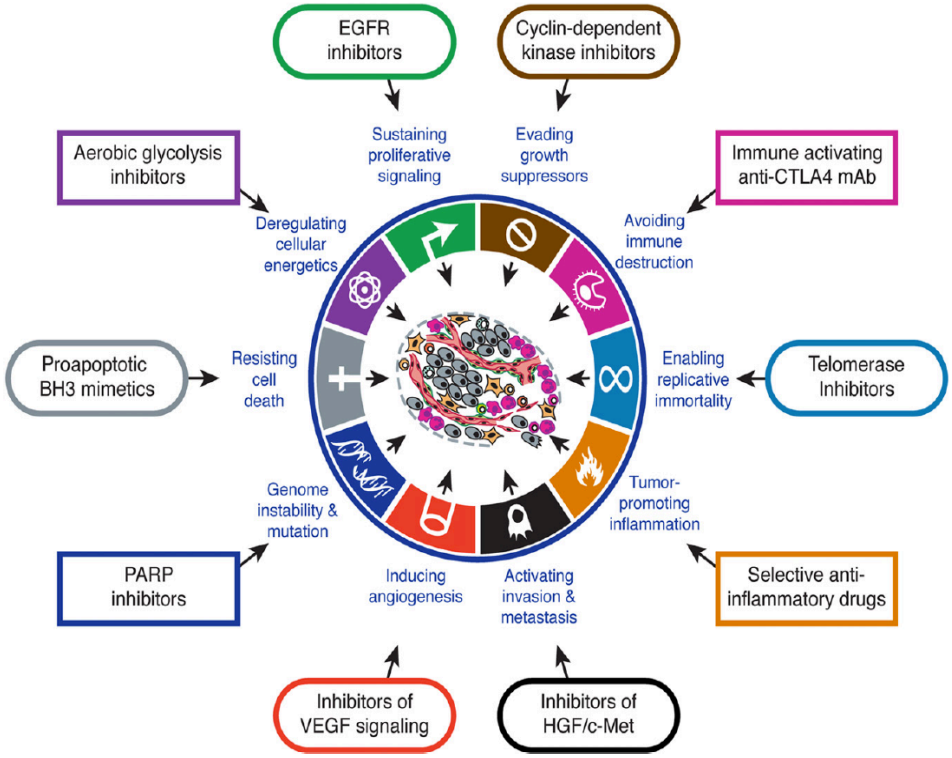
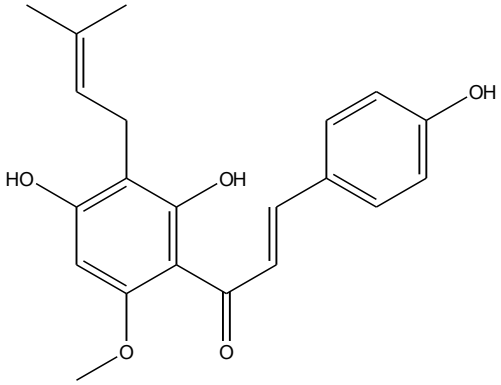


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

Inhibitors of angiogenesis

- **Curcumin**, the main curcuminoid of *Curcuma longa*, has been described as a direct **inhibitor of angiogenesis** in different cancer models.
  - Expression of proangiogenic factors (**VEGF, bFGF**) as well as cell adhesion molecules has been **decreased** upon curcumin treatment.
  - Mechanisms underlying its properties has been partially explained by its negative regulation of the transcription factors NF-kB, AP-1, protein kinase C (PKC) and mitogen-activated protein kinase (MAPK)

- Oral administration of **xanthohumol**, a constituent of *Humulus lupulus L.*, downregulated angiogenesis in dose-dependent manner *in vivo*.
  Significant reduction in **vessel formation** of this chalcone, has been linked to **inhibition of NF-kB and Akt** signaling



45 Xanthohumol

Humulus lupulus

From review: Orlikova and Diederich, submitted, 2011

Emerging hallmark: Avoiding immune destruction

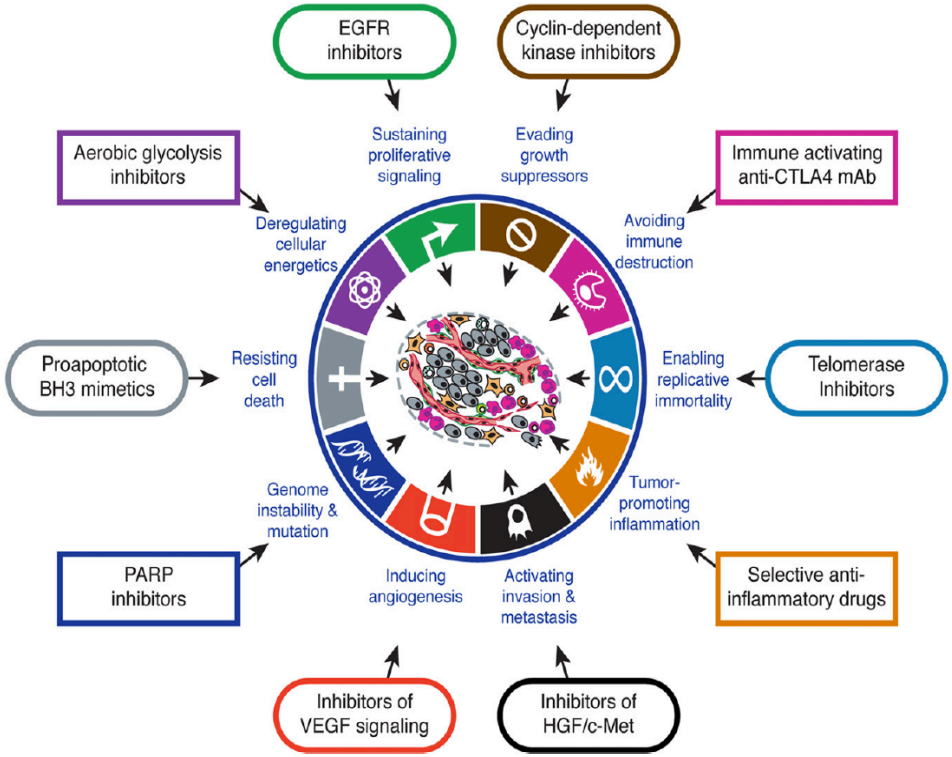


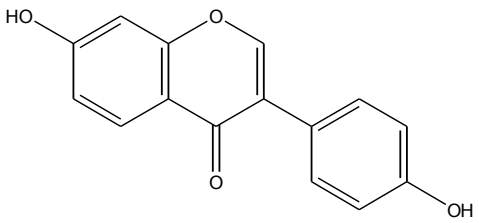
Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

Avoiding immune destruction

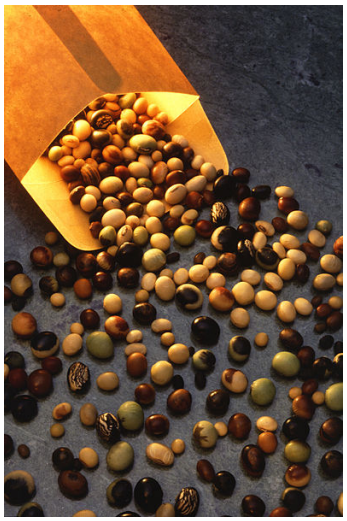
- One of the major functional capabilities of cancer is the ability of tumor cells to **evade immune destruction**.

- This emerging hallmark has been described recently and is improving our understanding how neoplastic tissue **favorizes growth and improves dissemination**

  – Soy isoflavone **daidzein**



24 Daidzein

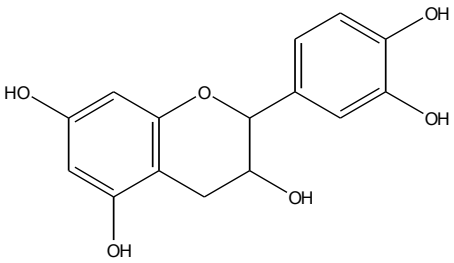


Glycine max

From review: Orlikova and Diederich, submitted, 2011

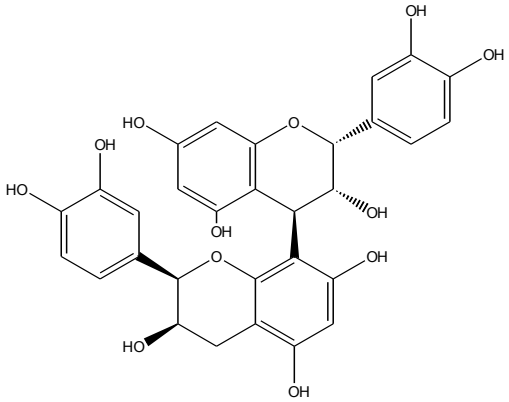
Avoiding immune destruction

- flavonoids extracted from *Litchi chinensis:*
  - epicatechin
  - proanthocyanidin B2
  - proanthocyanidin B4



25
Epicatechin

Litchi chinensis



26 Proanthocyanidin B2

From review: Orlikova and Diederich, submitted, 2011

Avoiding immune destruction

- **Extracts with immuno-modulatory** properties, which potentiate specific elements of the immune machinery and thus might promote enhanced **antitumor immunity**:

Glycyrrhiza glabra

Carpobrotus edulis

Euphorbia

From review: Orlikova and Diederich, submitted, 2011

Avoiding immune destruction

- **Extracts with immuno-modulatory** properties, which potentiate specific elements of the immune machinery and thus might promote enhanced **antitumor immunity**:

Salvia miltiorrhiza

Coriolus versicolor

Champia feldmannii

Panax gingseng

From review: Orlikova and Diederich, submitted, 2011

Emerging hallmark: Deregulated/activated cellular energetics
-> inhibitors of glycolysis, fatty acid metabolism

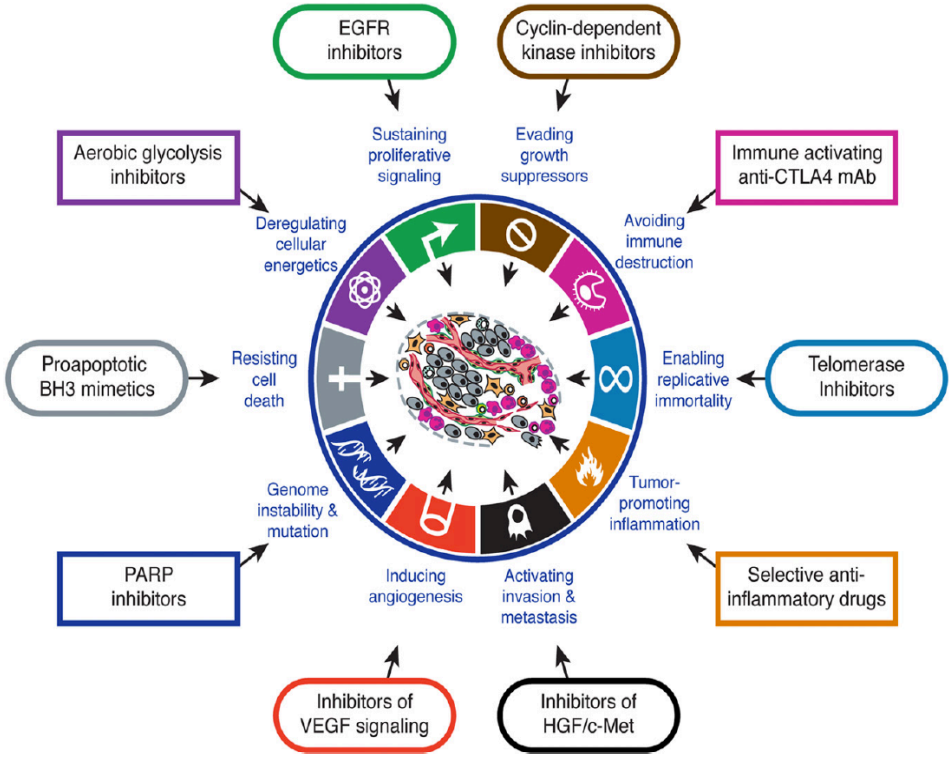
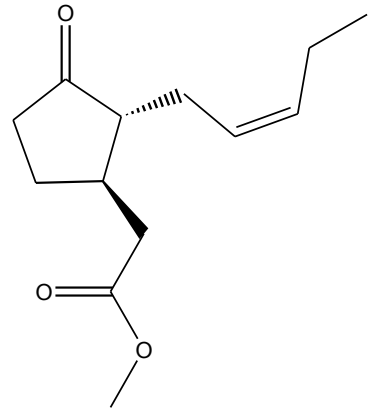


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

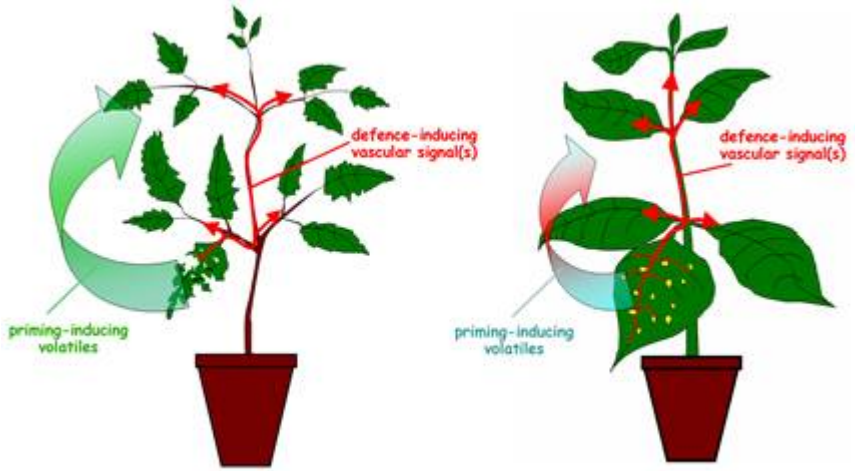
Inhibitors of glycolysis



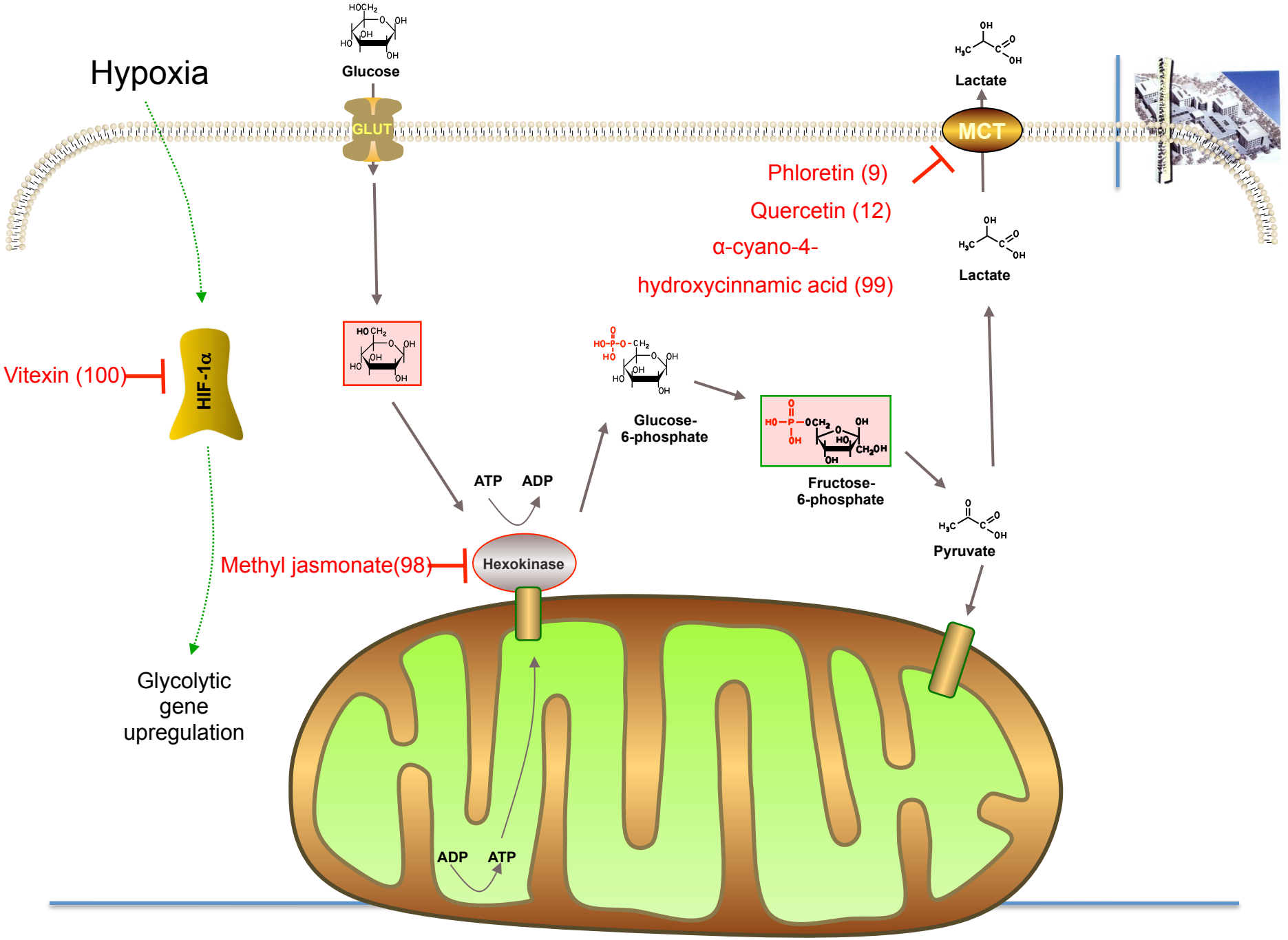
98 Methyl jasmonate

- **Hexokinase** is attached to **porins** in the outer mitochondrial membrane.
  - Destabilization of this junction will **prevent ATP release** from mitochondria.
  - Compounds attenuating alliance between hexokinase and porins will inhibit the glycolytic flux.

- **Methyl jasmonate, a plant stress hormone**, induced by plants as a chemical defense compound, has been identified as an active inducer of hexokinase release

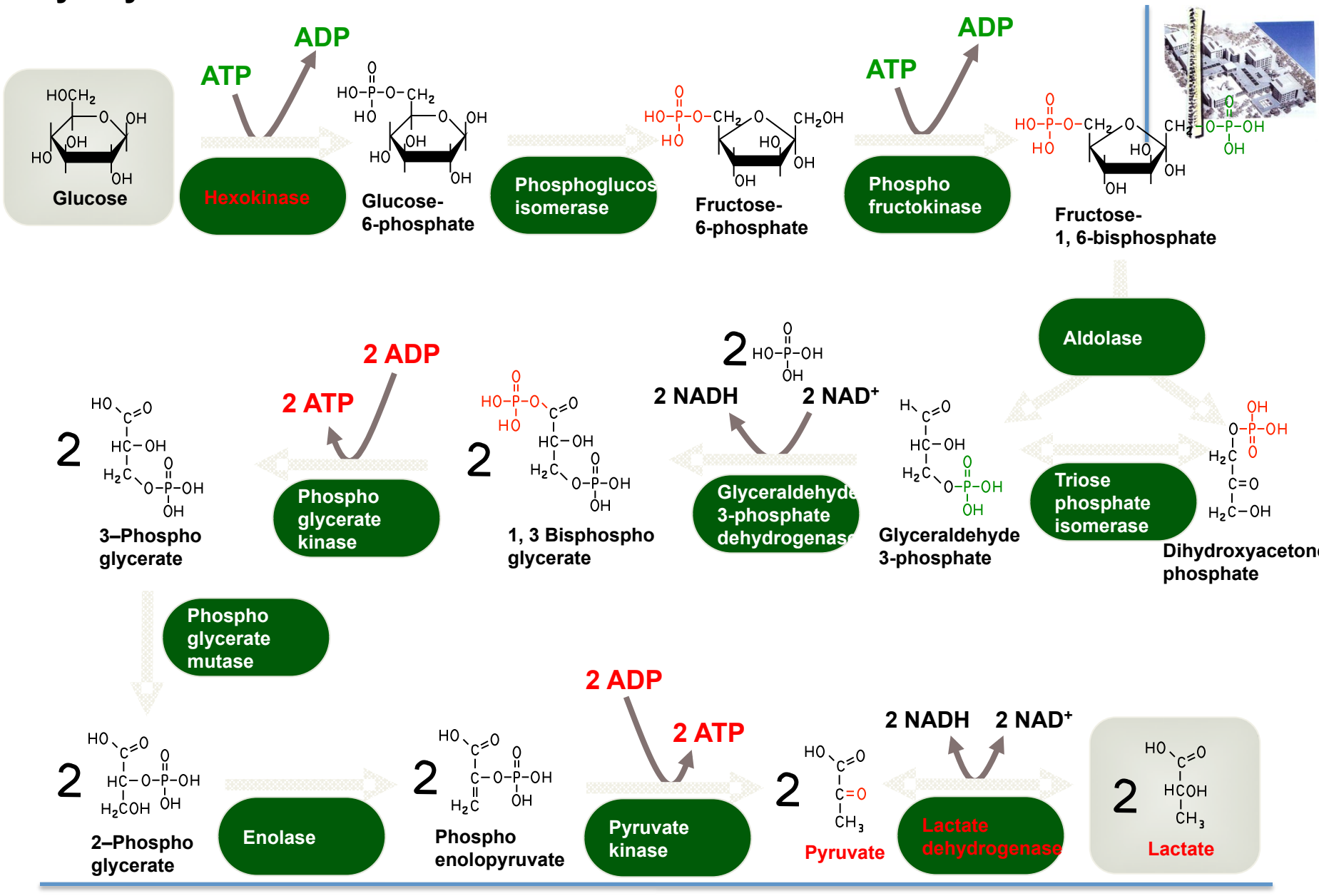


From review: Orlikova and Diederich, submitted, 2011



From review: Orlikova and Diederich, submitted, 2011

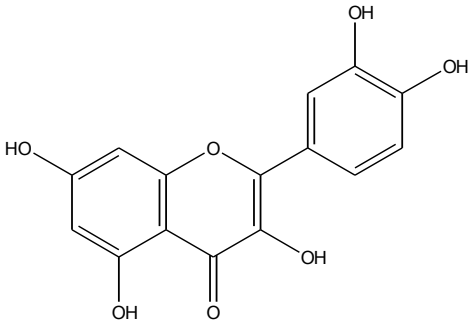
Glycolysis



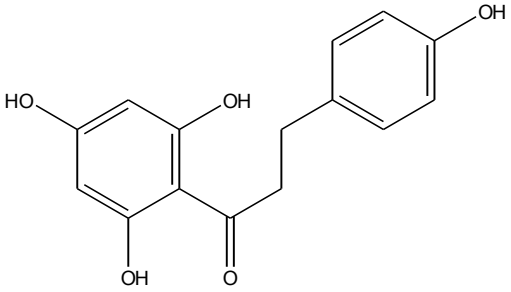
From review: Orlikova and Diederich, submitted, 2011

Inhibitors of glycolysis

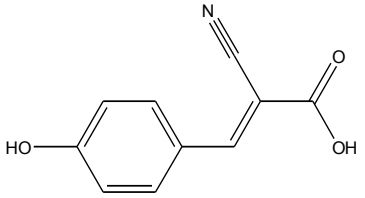
- The terminal stage of aerobic glycolysis is characterized by **pyruvate conversion into lactate** by lactate dehydrogenase and its subsequent release into the tumor microenvironment *via* transmembrane **monocarboxylate transporters (MCT).**
- **Disruption of MCT** function by small-molecule inhibitors either synthetic or of natural origin has already been reported:



12 Quercetin



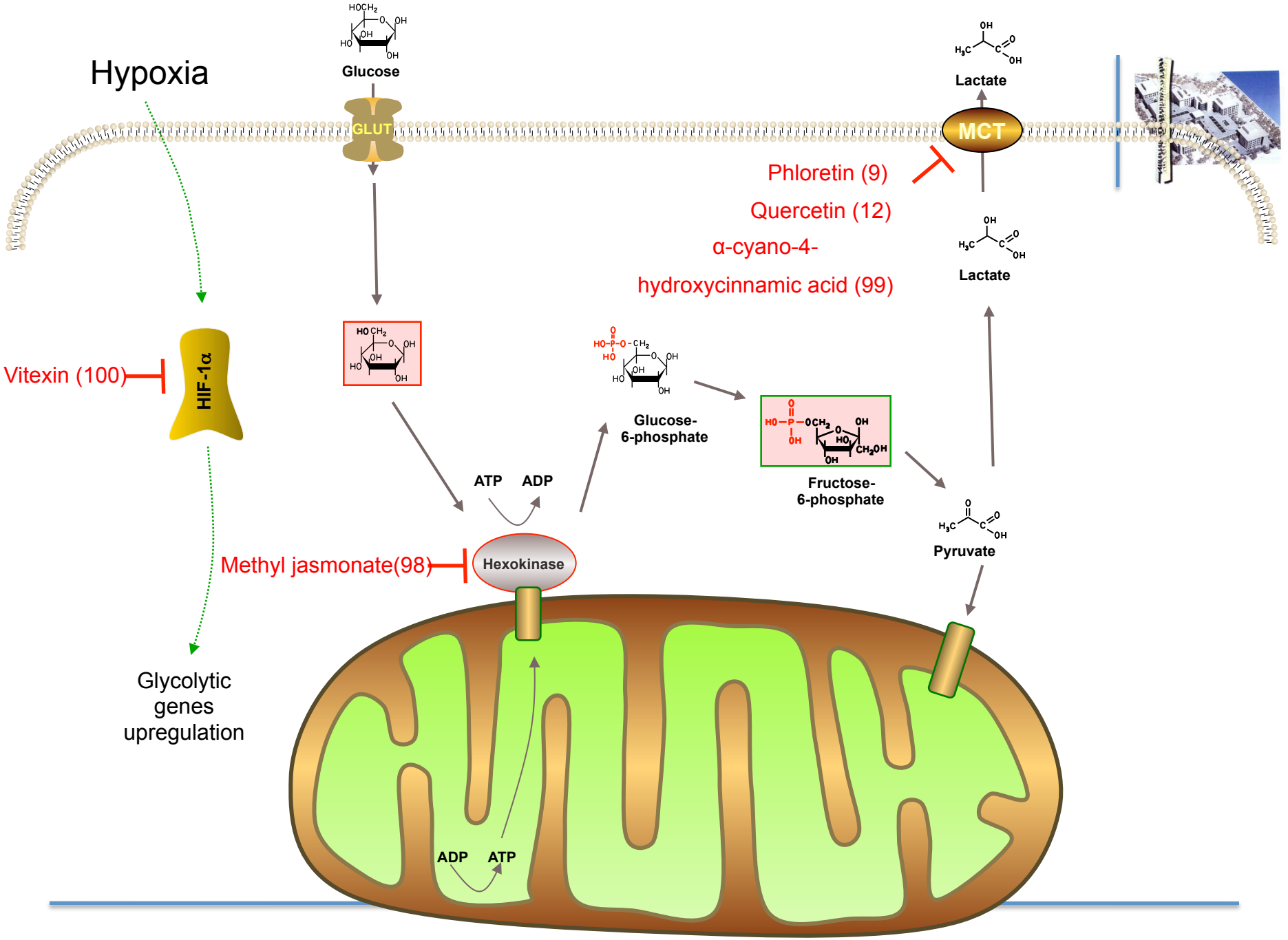
9 Phloretin



99 α-cyano-4-hydroxycinnamic acid

- **Blocking lactate release consequently abrogates pyruvate to lactate reduction in tumor, leading to metabolic crisis within the neoplastic tissue.**

From review: Orlikova and Diederich, submitted, 2011



From review: Orlikova and Diederich, submitted, 2011

Inhibitors of fatty acid metabolism

- Fatty acid metabolism is an additional attractive target within the dysregulated metabolism pathways in tumor cells.

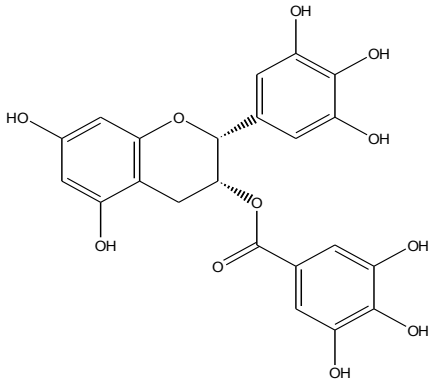
- De *novo* fatty acid synthesis is significantly **upregulated** in various types of cancer.

- **AMP-activated protein kinase (AMPK)** has been identified as a crucial metabolic regulator, **decreasing of the energetic state of the cell**

From review: Orlikova and Diederich, submitted, 2011

Inhibitors of fatty acid metabolism

- **EGCG** has a strong capability to induce AMPK



1 Epigallocatechin 3-gallate

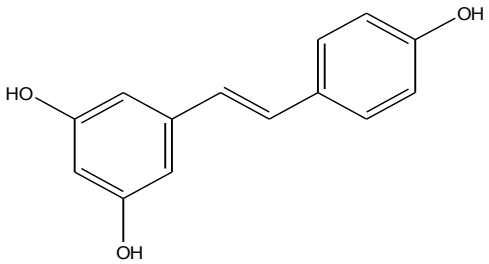
- **EGCG**-induced activation of AMPK was associated with a reduction of the glucose transporter Glut-1 and a proapoptotic effect in colon cancer.

From review: Orlikova and Diederich, submitted, 2011

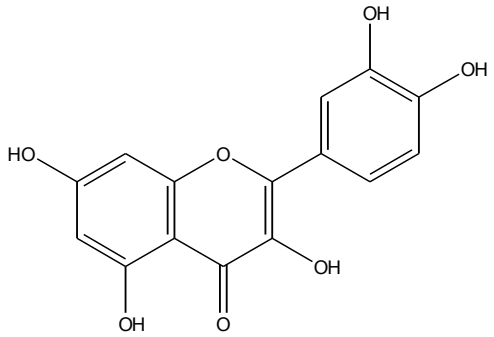
Inhibitors of fatty acid metabolism

- Increased levels of **fatty acid synthase (FAS)** in tumors are concomitant with the overall increase in endogenous fatty acid synthesis

- **Use of FAS inhibitors may lead to an increase of anticancer treatment efficiency**



41 Resveratrol



12 Quercetin

- Natural compounds such as **resveratrol** or **quercetin** have been shown to inhibit FAS

From review: Orlikova and Diederich, submitted, 2011

Hallmark: Resisting Cell death

Apoptosis

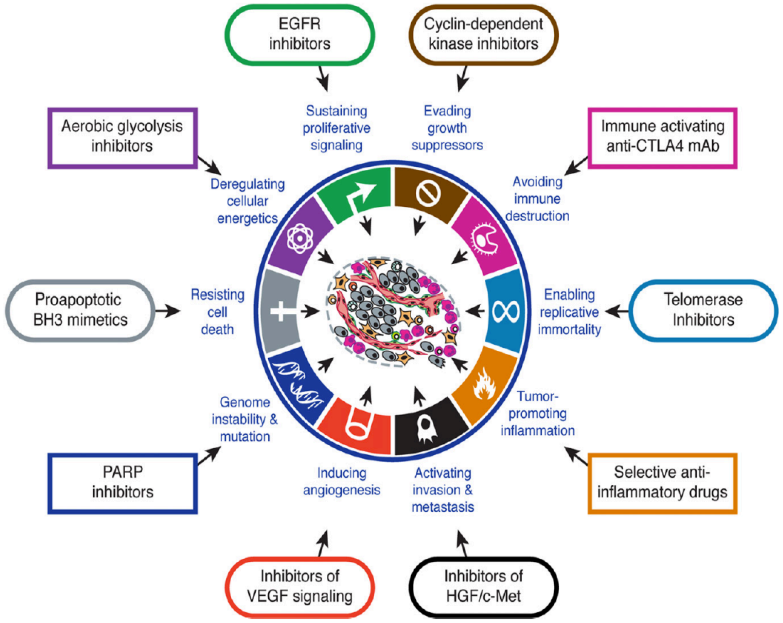


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer


Calotropis procera

Arka (*Calotropis procera*)

- **Arka is one of the herbs mentioned in all ancient scripts of Ayurveda.**
- **Dhanvantari Nighantu and Madanadi Nighantu have mentioned only two varieties of arka, namely : arka and alarka (rajarka)**
- **But Bhavaprakasa says that there is another variety called raktarka and describes its properties separately.**
- **The author of Raja Nighantu also enumerates three kinds of arka viz. arka, vetarka and rajarka and ascribes all of them separately.**

Aak is a Life Giver and Life Taker

- In India it was used in Vedic times in religious ceremonies, and the plant was considered sacred.
- The plant was among ancient nomadic Arab tribes.
- It has the power of giving both life and death.
- It was mentioned in Vedic medical texts dating back to 1500BC.

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Commentary

UNBS1450 from *Calotropis procera* as a regulator of signaling pathways involved in proliferation and cell death

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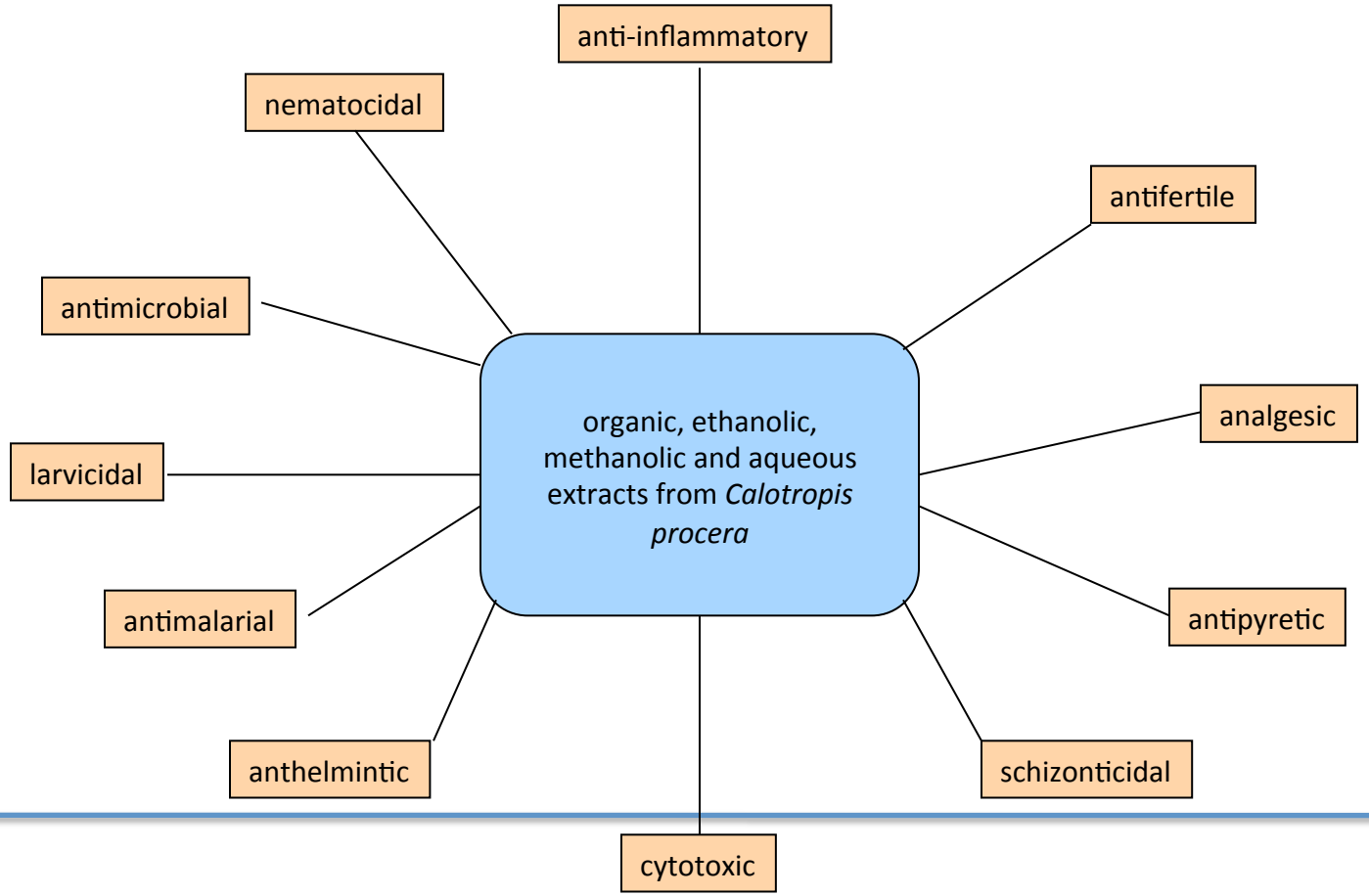
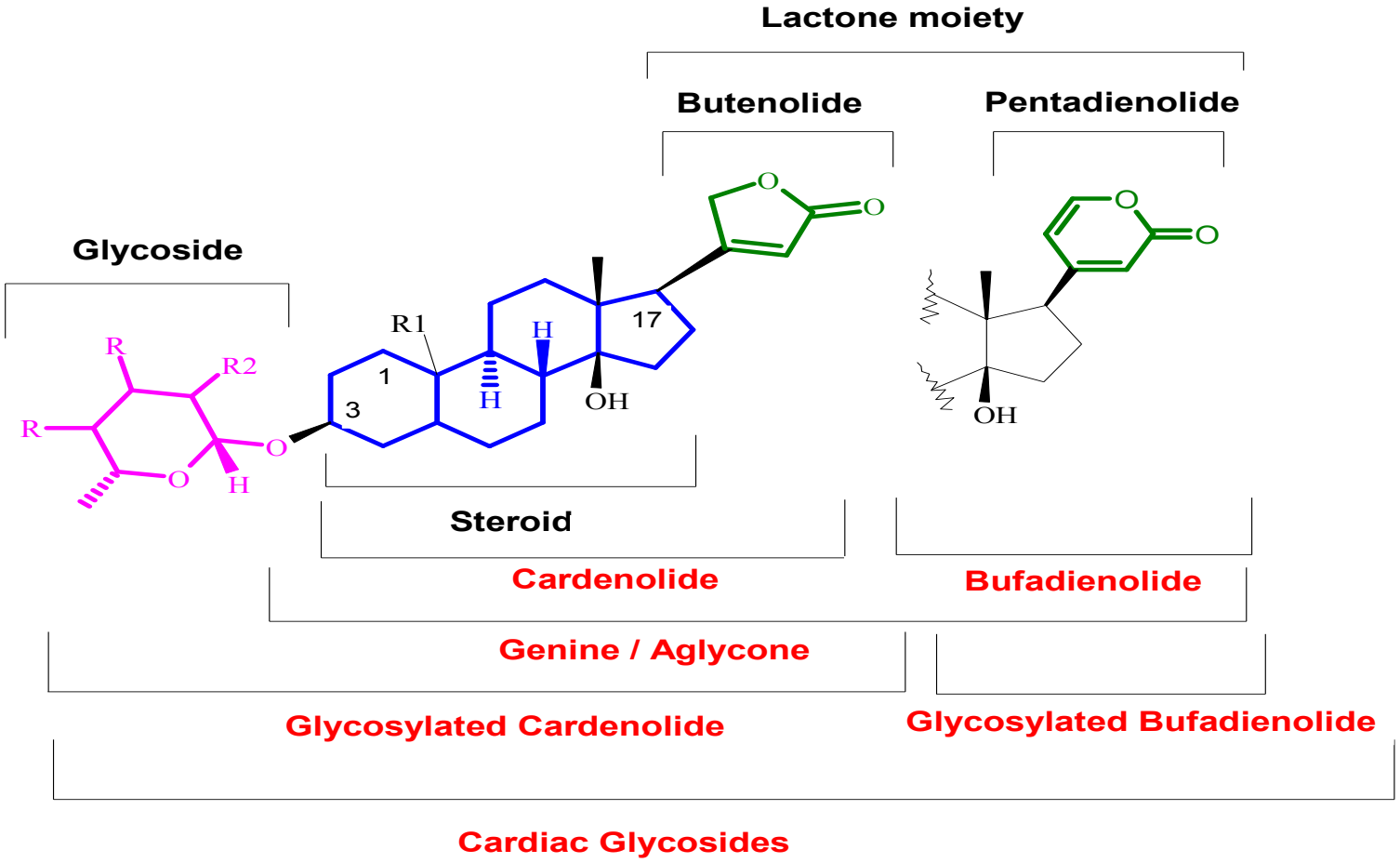


Structures of cardiac glycosides



Cardenolides are known since years for congestive heart failure treatment

Rationale for cardenolides in cancer

• **Epidemiological data :**

• **Lower mortality rates in cancer patients who were on digitalis at time of first diagnosis, compared to patients not on digitalis therapy :**

– **Stenkvist et al. (New England Journal of Medicine 1982) : 5y follow-up study :**

The recurrence rate of breast cancer in patients not on digitalis was 9.6 times higher than in patients treated with digitalis

– **Stenkvist et al. (Oncology Reports 1999) : 20y follow-up study :**

Death rate from breast carcinoma (excl. other causes of death & confounding factors) was 6% (2 of 32) among patients on digitalis (p=0.002)

– **Goldin & Safa (Lancet 1984) : retrospective study of 127 cancer patients :**

Of 21 deaths only 1 cancer death among those who had taken digitalis

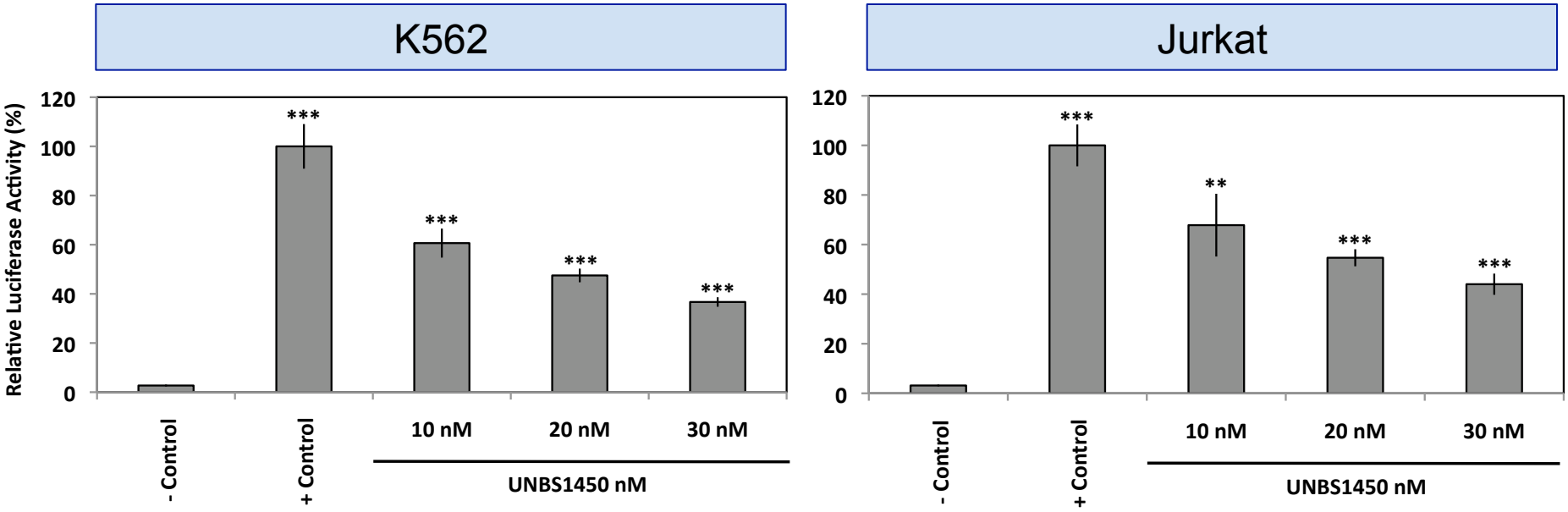
Rationale for cardenolide in cancer

- It was shown *in vitro* that cardiac glycosides inhibit malignant cell proliferation and have anti-proliferative and apoptotic effects in several cancer cell lines

- Differences characterize the potency of these structurally similar compounds: Johansson found that proscillaridin A was the most potent, followed by digitoxin, ouabain, digoxin, lanatoside C, digitoxigenin and digitonin

- UNBS1450 displayed better antitumor properties *in vitro* compared with commonly used chemotherapeutic drugs, and was best tolerated *in vivo* by mice compared with digitoxin and ouabain

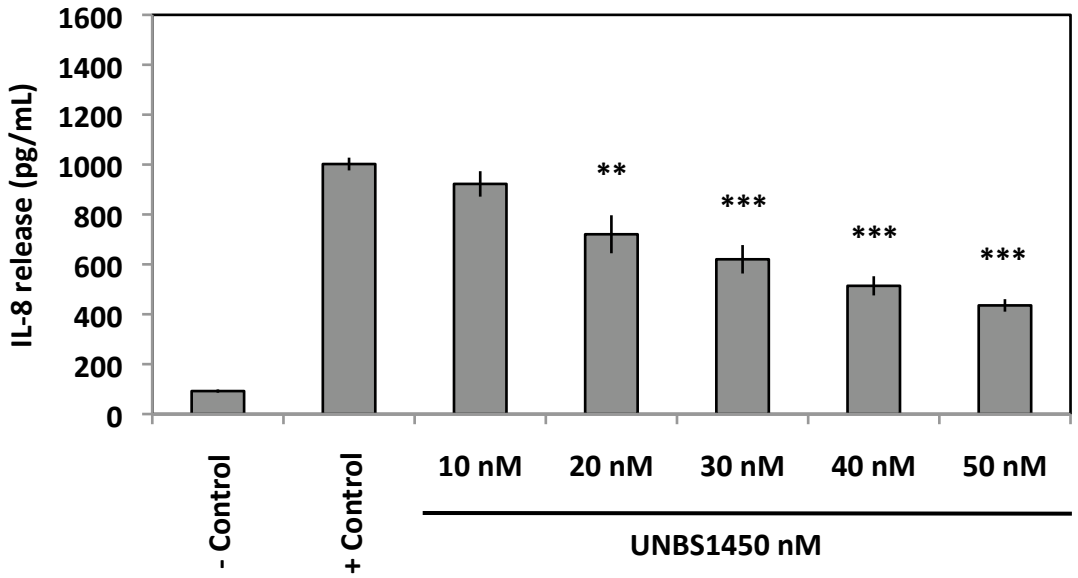
UNBS1450 represses TNFα-induced NF-κB activity



- ✓ UNBS1450 represses induced NF-κB transcription activity in both K562 (max. 63 %) and Jurkat (max. 55 %) cell lines
- ✓ dose-dependent inhibitory effect (≤ 30 nM)

Juncker et al, Biochem Pharmacol, 2011

Inhibition of Interleukin-8 production



Juncker et al, Biochem Pharmacol, 2011

Apoptosis induction as a therapeutic goal in cancer research

Intrinsic pathway (DNA damage, stress, virus)

Extrinsic pathway (TNF, FAS, TRAIL)

Apoptotic stimulus

p53

Bak

Bax

Bcl-2

Bcl-x_L

tBid

Bid

Bax

Bak

Cyt

SMAC

DIABLO

IAPs

APAF-1

Casp 9

Casp3

TRADD

FADD

Casp8

FLIP

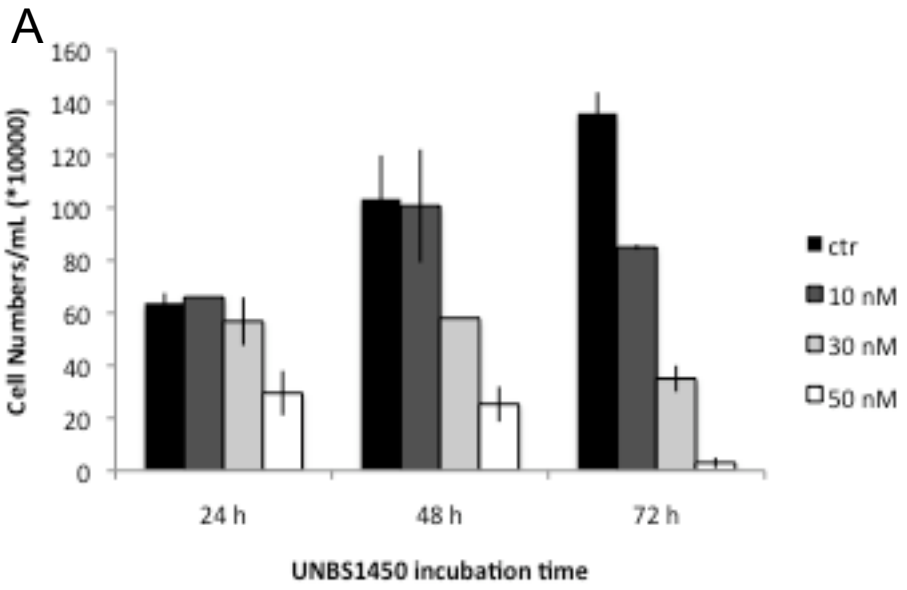
APOPTOSIS

Deregulation of various elements

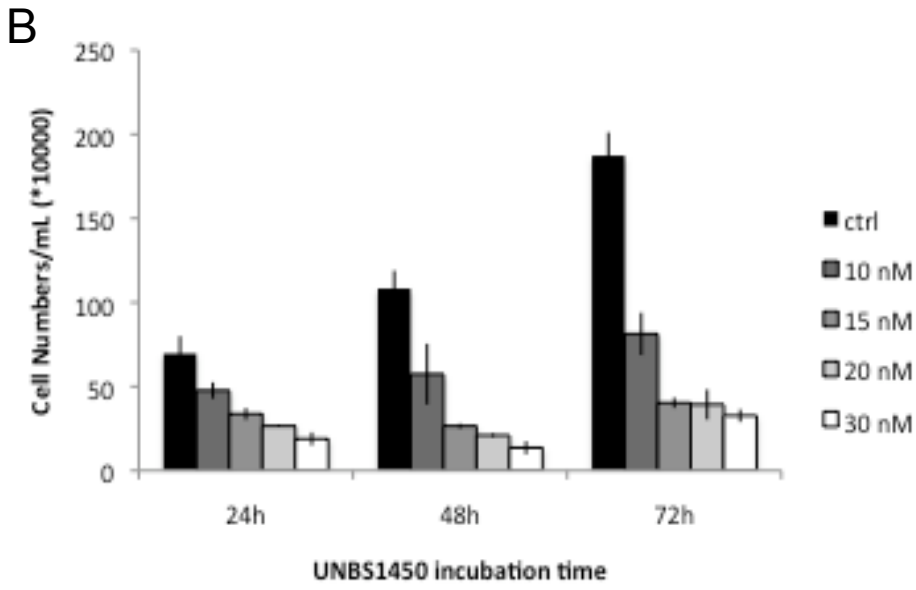
CANCER

Effects of UNBS1450 on cell proliferation

K562

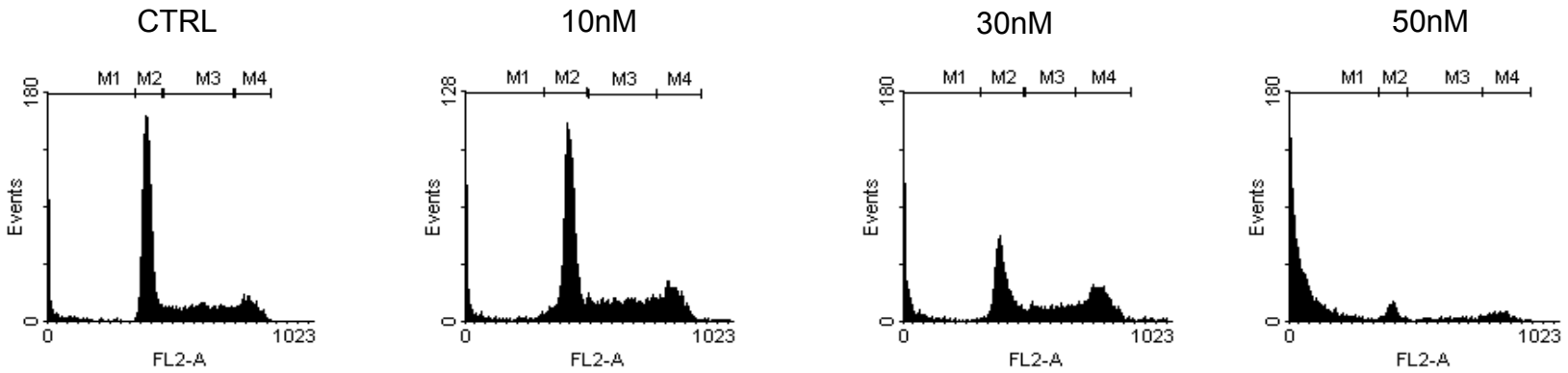


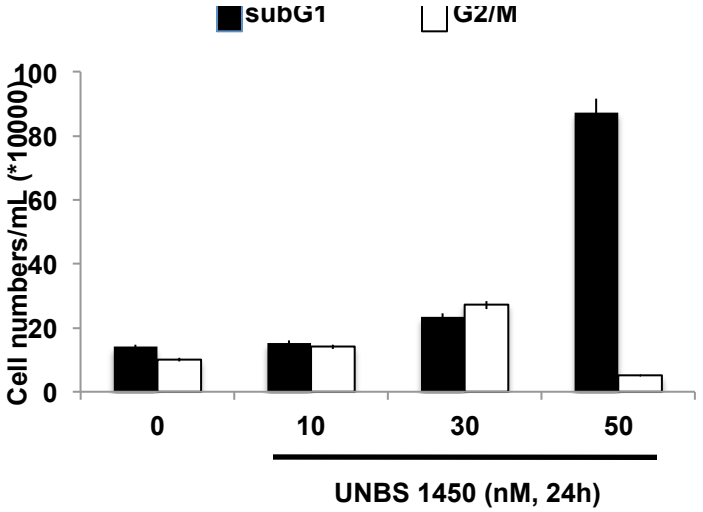
U937



Juncker et al, Biochem Pharmacol, 2011

Analysis of UNBS1450 effect on cell viability in K562 cells

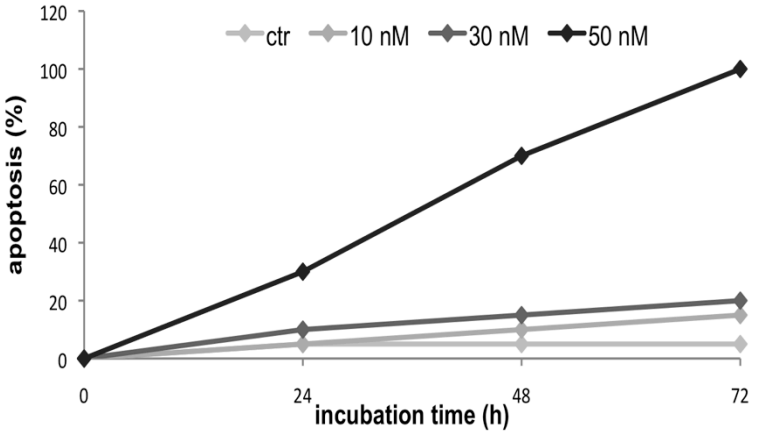




Juncker et al, Biochem Pharmacol, 2011

UNBS1450 induces apoptosis in chronic myeloid leukemia K562

A



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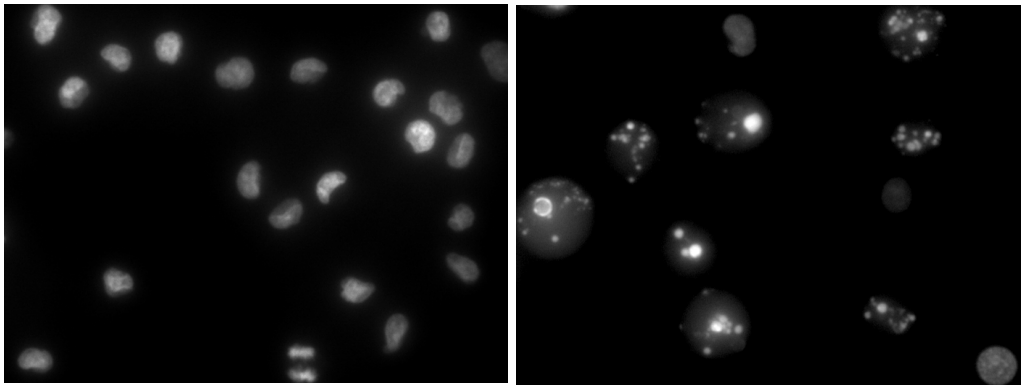

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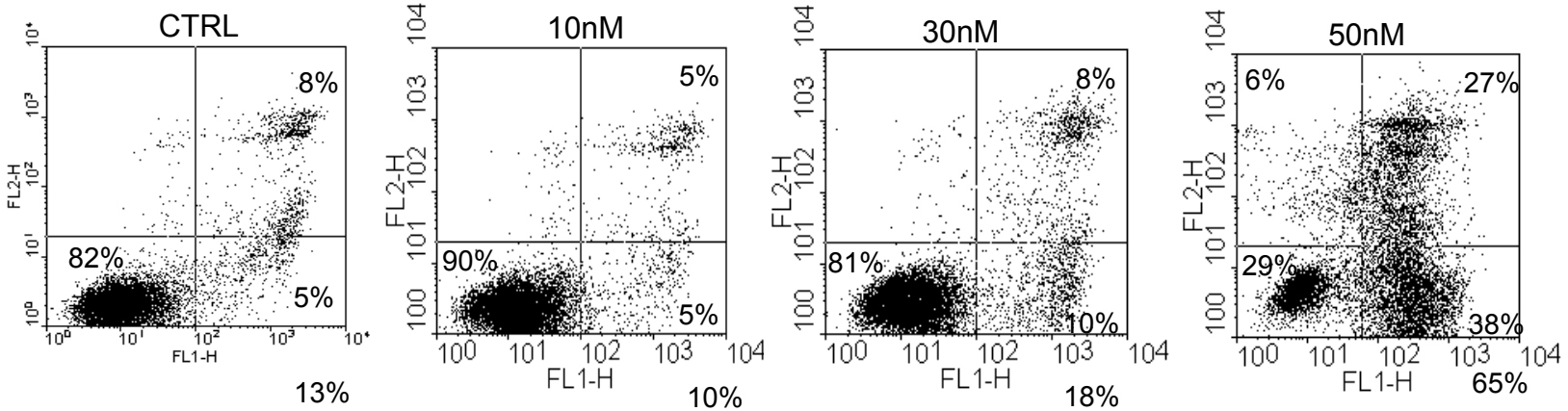

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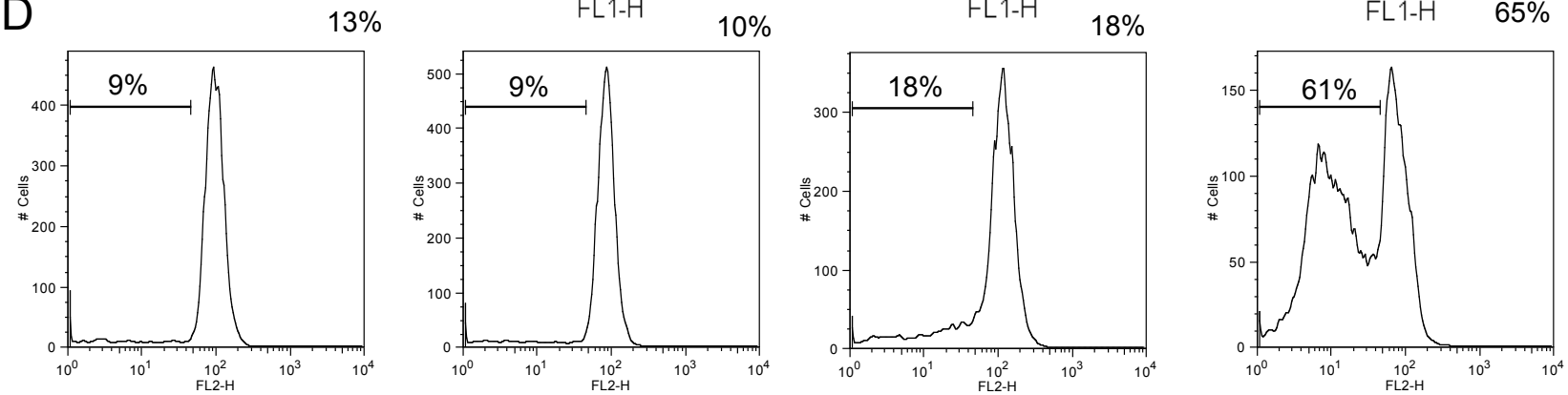
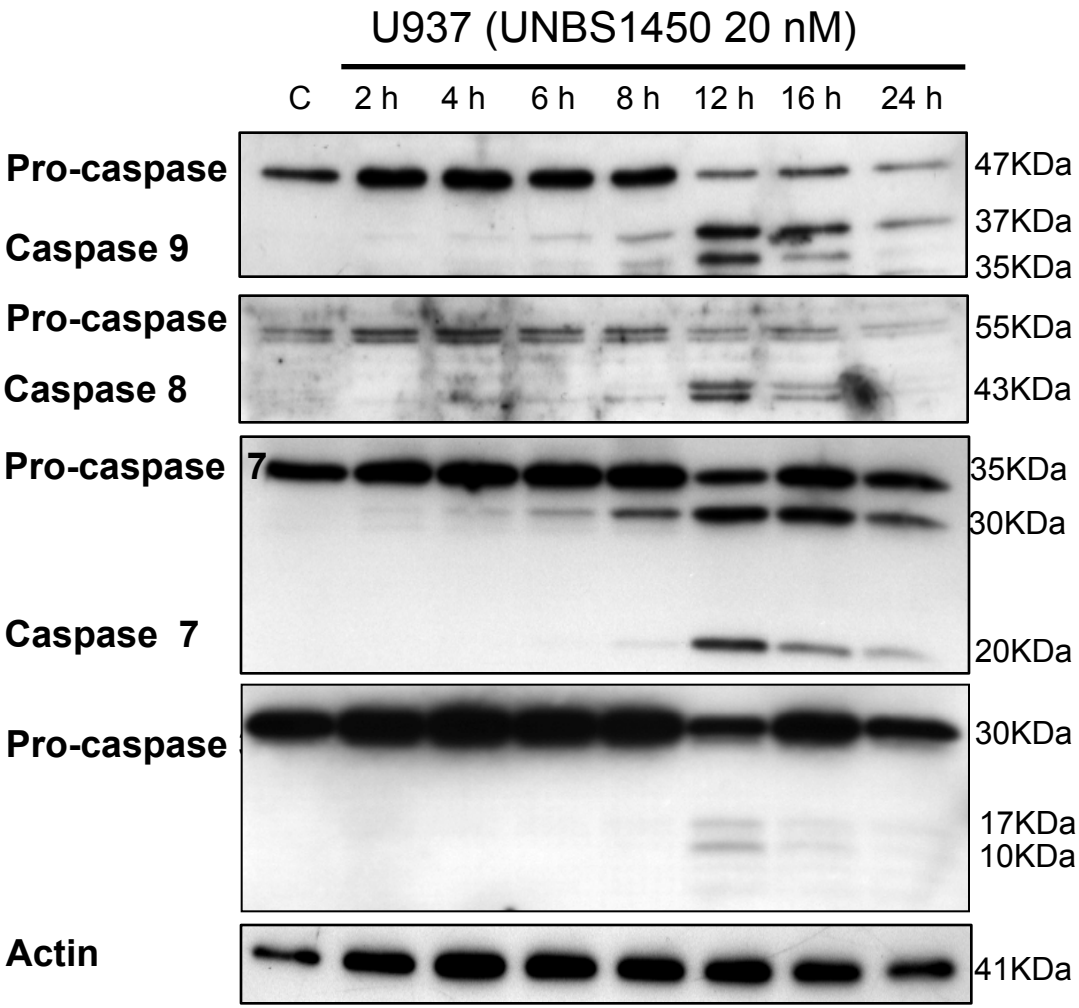


Juncker et al, Biochem Pharmacol, 2011

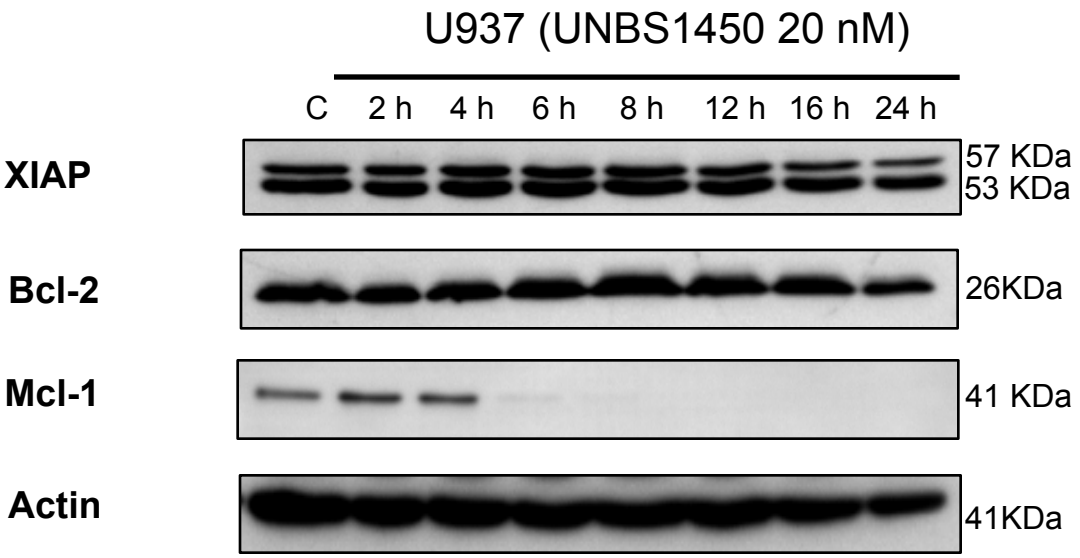
K562

Analysis of UNBS1450 effect on caspase activation in U937 cells



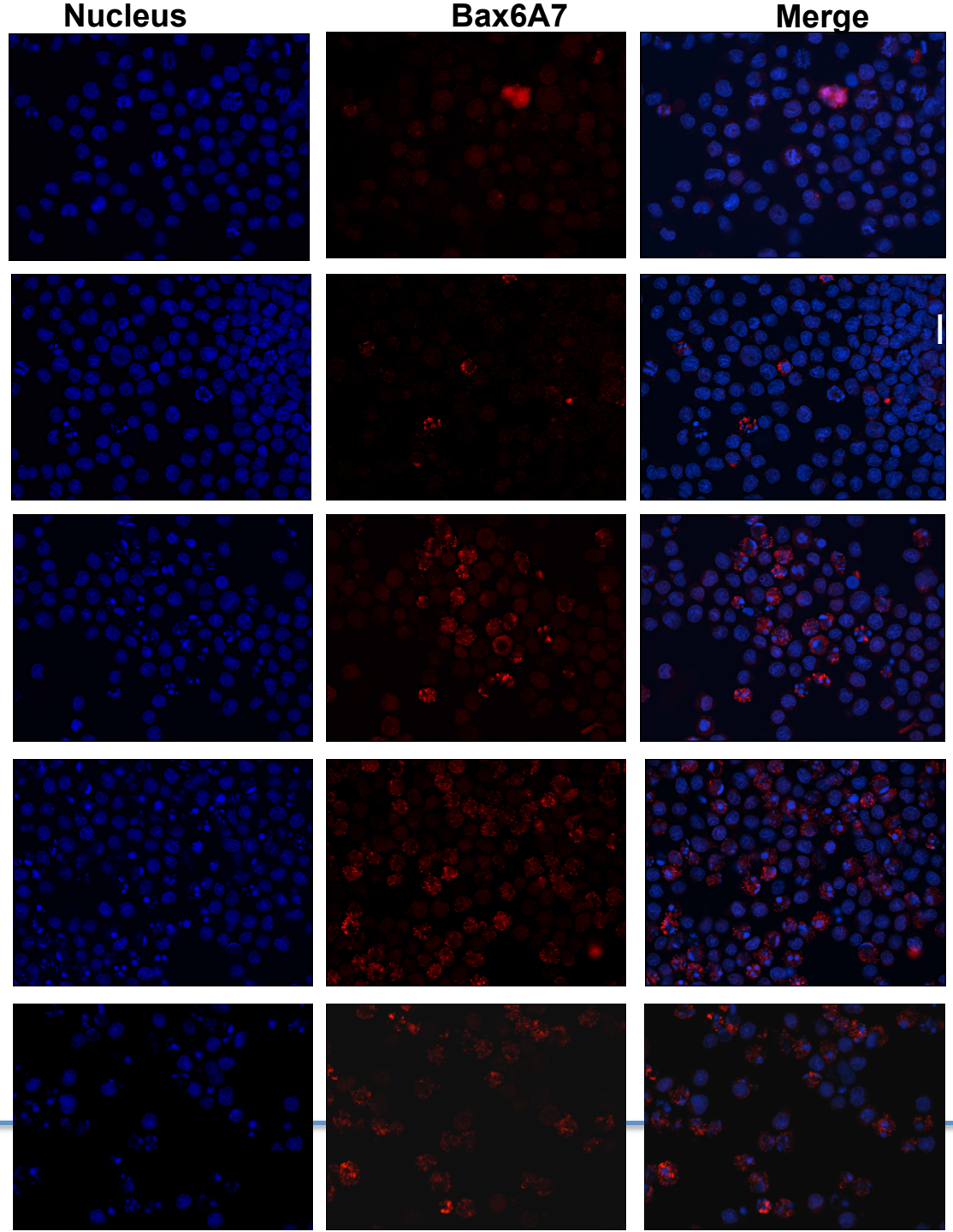
Juncker et al, Biochem Pharmacol, 2011

Analysis of UNBS1450 effect on anti-apoptotic proteins in U937 cells



Juncker et al, Biochem Pharmacol, 2011

A.



U937

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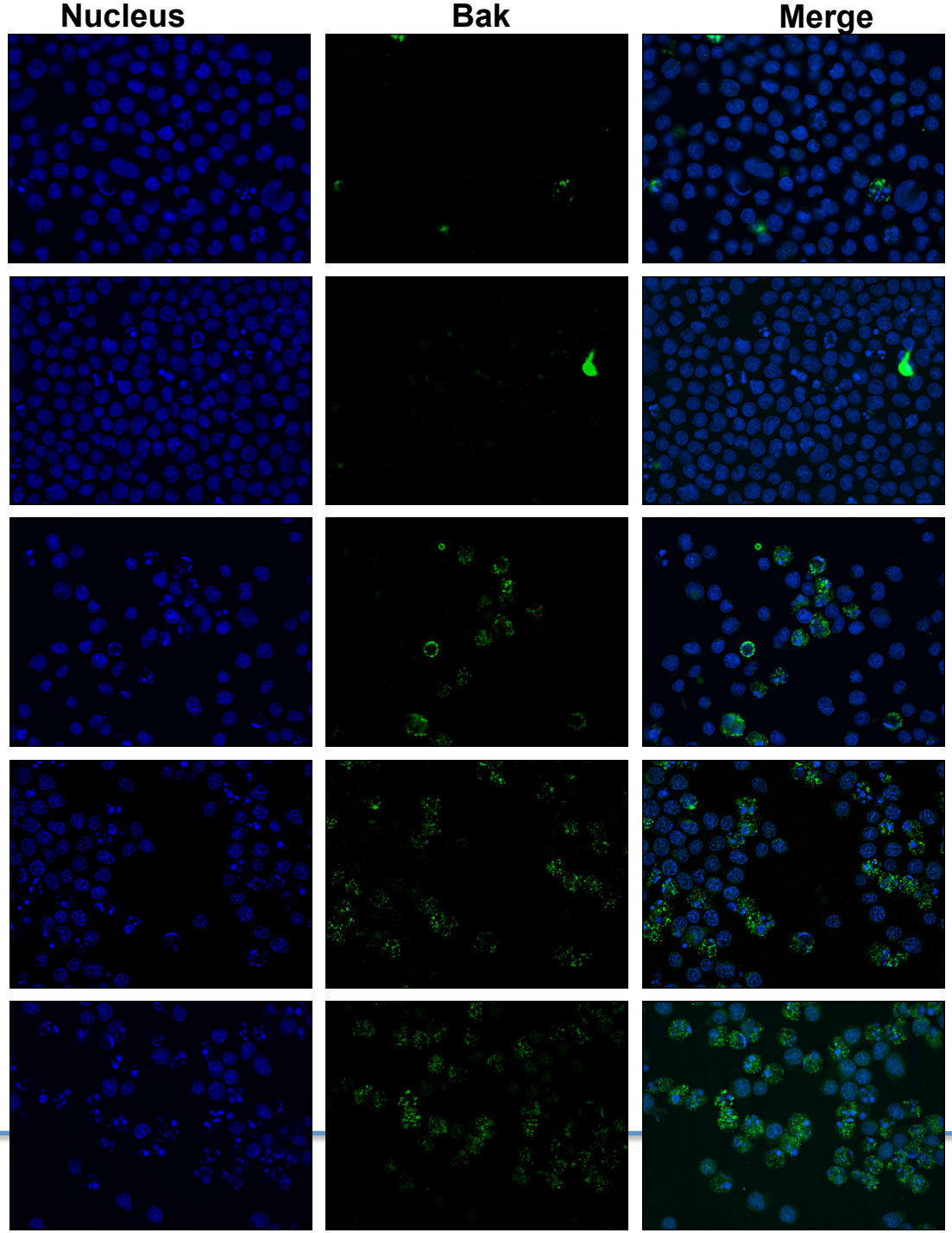
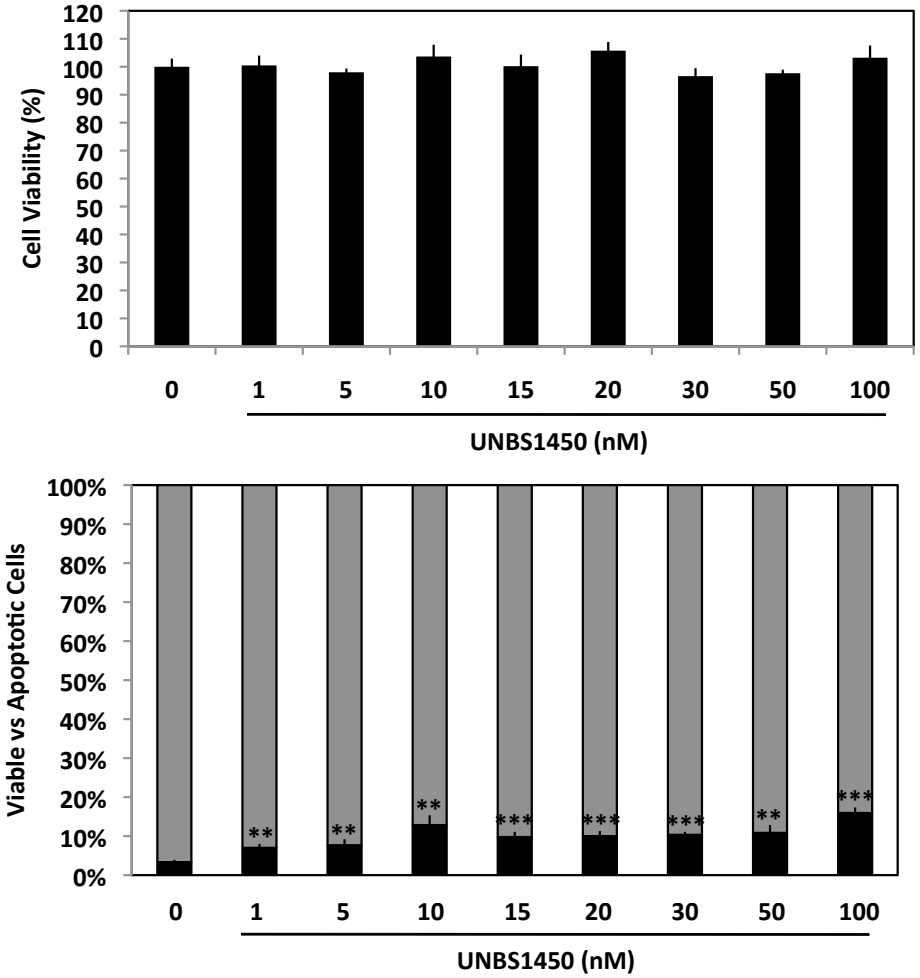
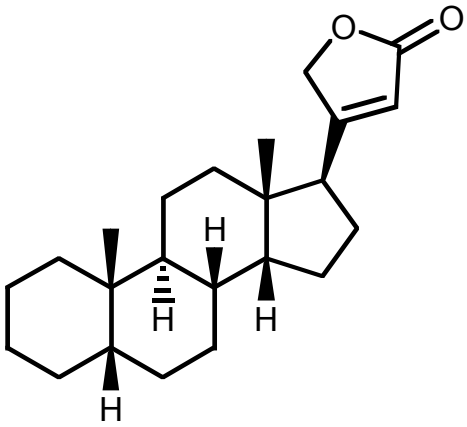


U937

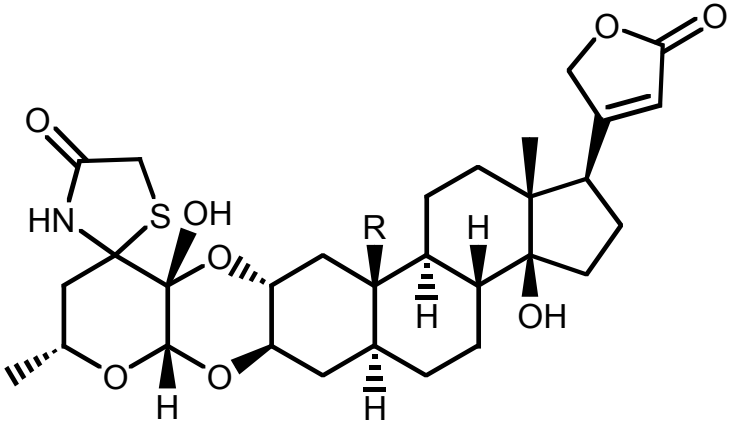
Differential toxicity of UNBS1450



Juncker et al, Biochem Pharmacol, 2011



Cardenolide structure



R=CH_2OH	UNBS1450 **01**
R=CHO	2’’-oxovoruscharin **02**

Juncker *et al.*, 2009

Rationale for UNBS1450 in cancer

- **FIRST-IN-CLASS compound:**
  - **UNBS1450 is a novel cardenolide in clinical development for oncology**
  - **New MoA : NCI COMPARE analysis reveals no correlation to standard agents or to other cardiac glycosides**

- **Higher *in vivo* anti-tumor activity in aggressive & metastatic orthotopic models (NSCLC, refractory prostate cancer, melanoma & diffusely invasive glioma in nude mice) than taxol, irinotecan, oxaliplatin, mitoxantrone, temozolomide, etc.**

- **Impressively active in multi drug resistant cancer cells whether conferred by over-expression of drug-transporter proteins or induced by chemotherapeutic agents**

Ongoing phase I study

- Population is cancer patients with solid tumors and lymphoma
- Standard Dose escalation design 3+3
- A single administration every three weeks (1h IV infusion)
  - to determine the dose limiting toxicities & maximum tolerated dose
  - and look for early evidence of antitumor activity
- Phase I study is open at 2 clinical sites in Europe
  - » Leiden University Medical Center (Dr. Hans Gelderblom)
  - » Leuven University Hospital Gasthuisberg (Dr. Patrick Schöffski)
- 21 patients treated, enrolling in 7th dose cohort
- (615 µg/patient)

UNBS1450 : Enrollment history

Dose (µg/patient)	# patients	# cycles	Average # cycles	# active
90	3 - Melanoma, colorectal, colon	2,2,1	1.6	0
140	3 - Esophageal, colorectal, pancreatic	2,3,7	4.0	0
210	3 - Colorectal, adenoid cystic, sarcoma	7,6,2	5	0
265	4 – Chondrosarcoma (replaced), melanoma, colon, colon carcinoma	1,2,2,1	1.5	0
350	3 – Melanoma, colorectal, neuroendocrine	3,2,3	2.7	0
465	4 - Melanoma, chondrosarcoma, uterus sarcoma (replaced), melanoma	1,4,1,1	1.75	0
615	1 - Biliary	1	1	1

UNBS1450 : Clinical trial

- **Safety update**
  - **No DLTs (Dose Limiting Toxicities)**
  - **No drug related SAEs/SUSARs**
    - **SAE/SUSAR reported : Pulmonary embolism**
    - **SAE reported : Pain, nausea, vomiting, constipation**
    - **SAE reported : Progressive disease**
  - **Cardiac events**
    - **1st degree asymptomatic A-V heart block by ECG**
    - **Bradycardia and tachycardia**
    - **T-wave changes**
  - **Other safety events**
    - **One patient in the 3rd dose (210 ug) developed drug-related grade 2 neuropathy**
    - **One patient in the 5th dose (350 ug) developed transient blurred vision**

- **Antitumor activity not yet observed though several patients with prolonged stable disease have been observed**

Preliminary Analysis of Treatment-Related Adverse Events (AEs) Regardless of Cycle

Phase I Study of UNBS1450 (n = 18; through 465µg)

	Number of Patients with AE			
	Grade 1	Grade 2	Grade 3	Grade 4
Anemia		1		
Anorexia	2			
Blurred Vision	1			
Conjunctivitis	1			
Diarrhea			1	
Dyspepsia	1			
Fatigue	1	1	1	
Fever	1			
Hair Discoloration	1			
Neuropathy (lower)		1		
Neuropathy (upper)		1		
Pain	1			
Vomiting	1			
Weight Loss	1			

Options moving forward
Phase II single agent studies

- **Selected solid tumor indications**
  - **Tumor indication based on hierarchical approach**
    - **Potential targets include melanoma, colorectal and NSCLC**
    - **Consider small orphan (*i.e.*, child brain tumor, Diederich lab) high unmet medical need indication to support rapid approval**
  - **Enrollment requires investigation of role of sodium pump overexpression**

- **Hematologic malignancies (Diederich lab)**
  - **Leukemia including AML**
  - **NHL**

Options moving forward Increasing exposure time in leukemia

- **Preclinical efficacy data with UNBS1450 :**
  - **Marc Diederich – Leukemia study**
  - **In vitro cytotoxicity studies had prolonged (i.e. 24-72 hour) exposures**
  - **UNBS1450 has potent activity in leukemia in the range of 15-30nM**
  - **In vivo xenograft studies had multiple doses per week**
- **PK results show that UNBS1450 has a very short mean $T_{1/2}$ of ~10 min**

Translate medicinal traditions into molecular mechanisms and pharmaceutical use: UNBS 1450

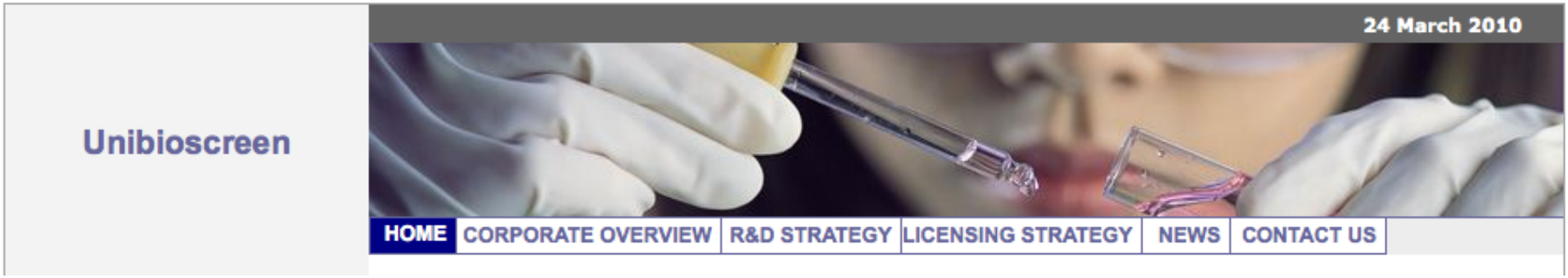
TOXICOLOGY STUDIES → Notox

FORMULATION & PRODUCTION →
- API : Novasep
- Drug Formulation : Notox
- Drug Product Manufacturing : Nerviano

IMPD REGULATORY →
- IMPD submitted
- Approval from Competent Authorities in Nl & B
- Approval from Ethical Committees of both sites

CLINICAL PHASE I →
- PI : Dr Patrick Schöffski & Dr Hans Gelderblom
- Sites : UZ Gasthuisberg & Leiden Hospital

Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
2007		2008				2009			



UNIBIOSCREEN

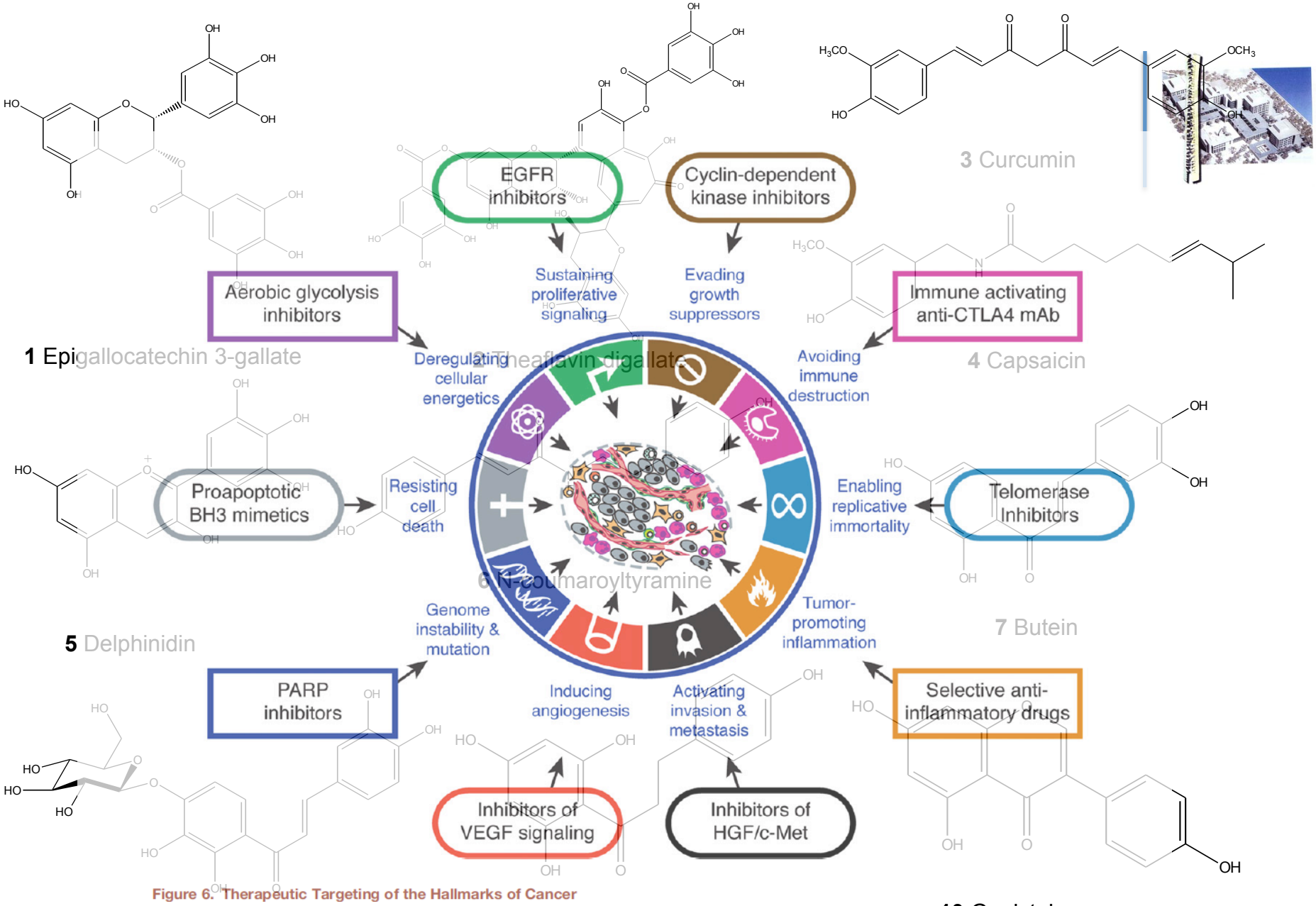


Figure 6. Therapeutic Targeting of the Hallmarks of Cancer

1 Epigallocatechin 3-gallate

2 Theaflavin digallate

3 Curcumin

4 Capsaicin

5 Delphinidin

6 N-coumaroyltyramine

7 Butein

8 Marein

9 Phloretin

10 Genistein

Financial contributions …

Fondation Recherche Cancer & Sang

RSL Recherches Scientifiques Luxembourg

télévie

LIONS

Fonds National de la Recherche Luxembourg

fnrs LA LIBERTÉ DE CHERCHER

M.C. MINISTERE DE LA CULTURE

EUROPA DONNA Luxembourg

UNIBIOSCREEN and investors

Groß / Grande REGION

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Programme opérationnel de coopération transfrontalière INTERREG IVA « Grande Région »
Europäische Territoriale Zusammenarbeit 2007-2013
Grenzüberschreitendes Programm zur Europäischen Territorialen Zusammenarbeit „Großregion“

MARIE CURIE ACTIONS

REDCAT

SEVENTH FRAMEWORK PROGRAMME

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