APPLICATION OF COMPUTERS, BIOINFORMATICS, AND MODELING TO PREDICT EFFECTIVENESS AND SAFETY OF DIETARY SUPPLEMENTS

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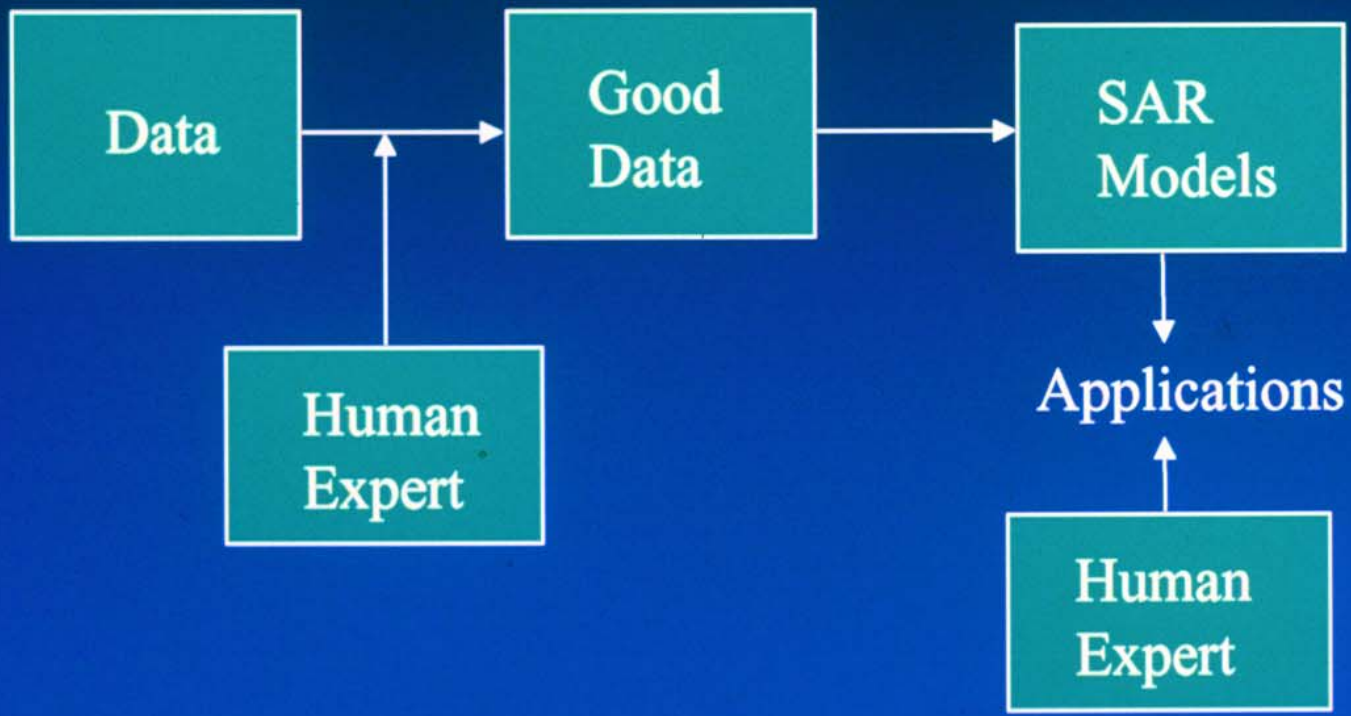
Experimental Data + Chemical Structure → SAR Model

SAR Model: Validation
Prediction and Documentation
Mechanism
Human Expertise

CASE/MULTICASE SAR Technology



SAR and Dietary Supplements

Cancer Chemoprevention (Many Assays)

Protein Kinase C Inhibition

COX-2 Inhibition

Antioxidants

Phosphatidylinositol 3-Kinase Inhibition

Tests Used To Ascertain The Ability To Prevent Cancers

A: inhibition of the induction of mutations in *Salmonella* caused by 4-nitroquinoline-N-oxide

B: inhibition of mammary tumors induced by DMBA

C: inhibition of Benzo[a]pyrene (B[a]P)-DNA binding

D: inhibition of TPA-induced tyrosine kinase activity,ornithine decarboxylase activity and free radical formation

E: inhibition of morphological transformation

F: inhibition of anchorage independence

G: induction of Phase II enzymes

H: inhibition of AOM-induced aberrant crypt formation

B.P.Thampatty[2001]

The molecule contains the Biophore (nr.occ.= 1):

```
-OH  -c              c   -C"
       \\          //
         cH  -cH
```

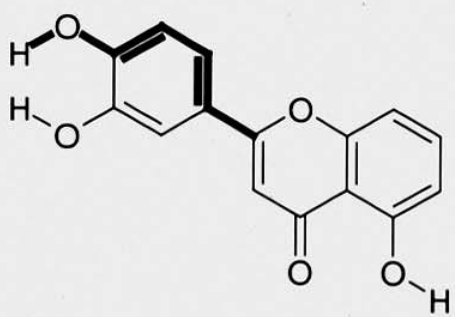
*** 6 out of the known 6 molecules (100%) containing such a Biophore
are COX-2 Inhibitors

*** QSAR Contribution : Constant is -18.55
** The following Modulators are also present:
Log partition coeff.= 3.90; LogP**2 contribution is 2.62
Ln Nr.Bi/Mol.Wt. = -5.60; Nr.Bioph/MW contrib. is 77.78

** Total projected QSAR activity 61.85

*** The probability that this molecule is a COX-2 Inhibitor is 87.5% **

** The projected COX-2 Inhibitory Activity is 61.9 CASE units **



The predicted COX-2 inhibiting activity of 5, 3', 4'- trihydroxyflavone

Predicted COX-2 Inhibiting Activity of Flavonoids

Chemical	Biophores B1 (2D)	B2
CATECHIN	---	---
CHALCONE	---	---
5,7-DIHYDROXY-4'METHOXYISOFLAVONE	---	62u
CHRYSIN	53u	---
FISETIN	62u	61u
FLAVANONE	---	---
FORMONONETIN	44u	---
HESPERETIN	53u	---
4'-HYDROXYFLAVONE	---	50u
2'-HYDROXYGENISTEIN	44u	62u
ISOFLAVONE	---	---
ISORHAMNETIN	71u	63u
KAEMPFEROL	44u	52u
MORIN	44u	62u
QUERCETIN	71u	62u
SILYBARIN	53u	
TANGERETIN	---	---

Example of Drug Discovery and/or Design

Dietary Supplements

Long Term Administration

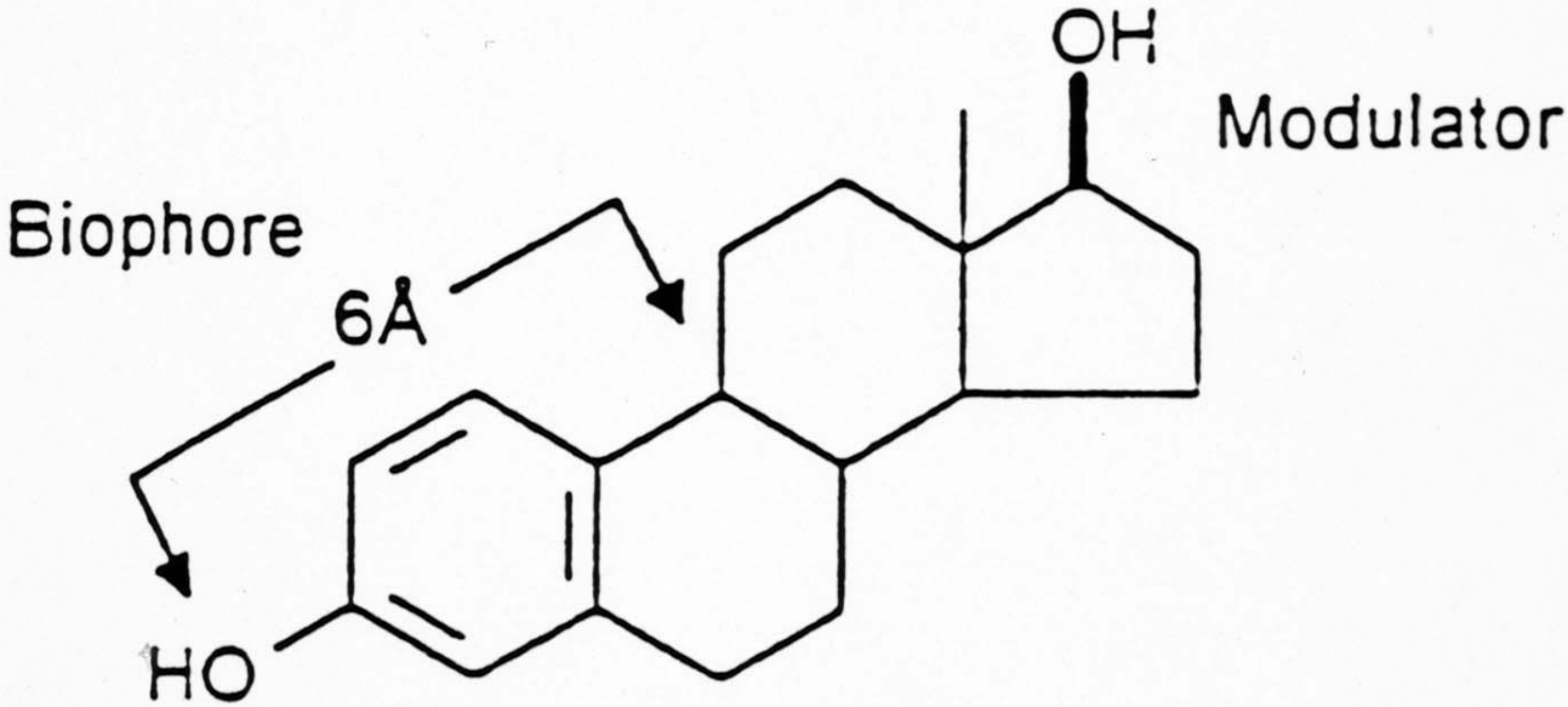
Benefits ???

Must be Risk-free

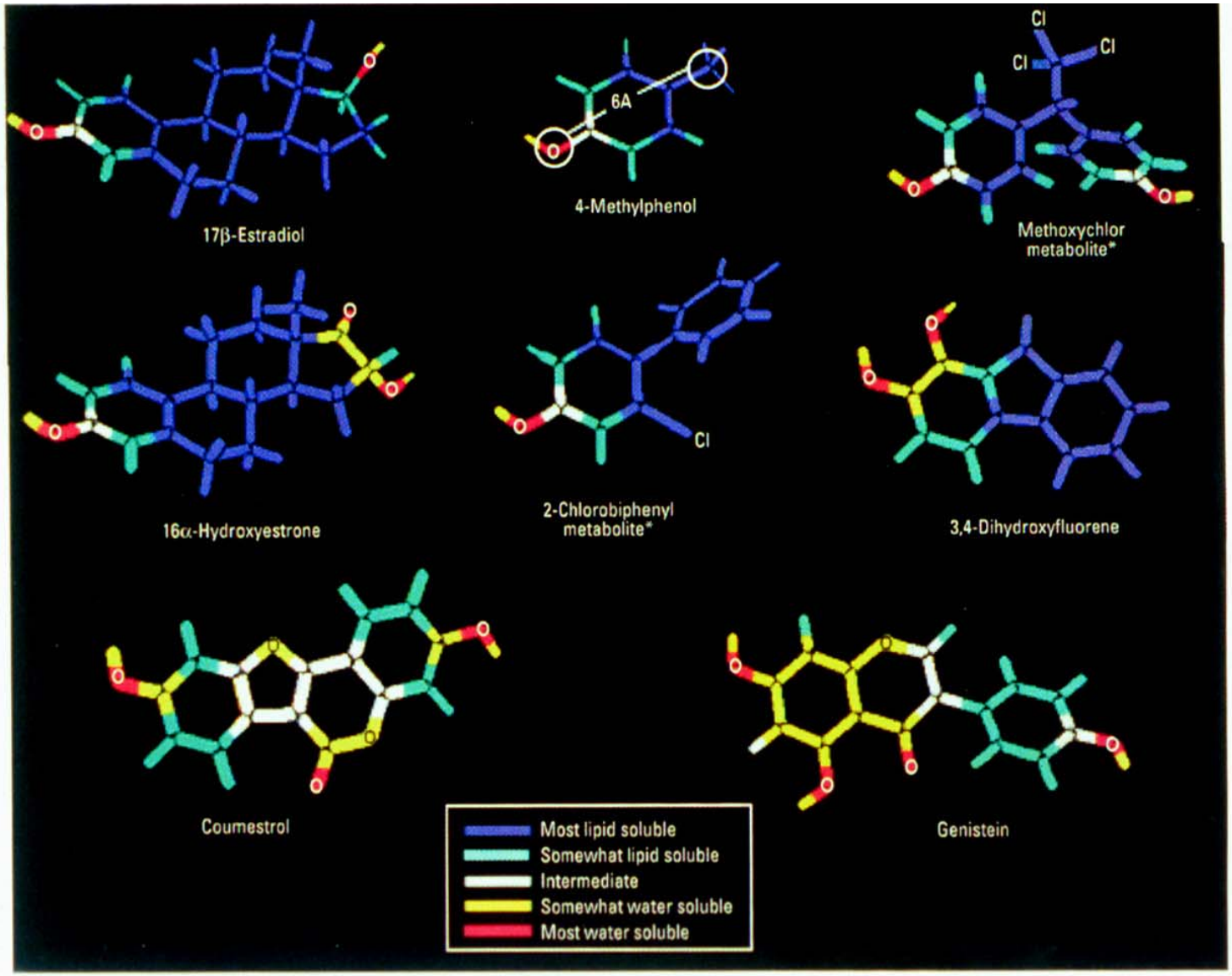
SAR Profiling

Hazard Identification

SAR Analyses of Carcinogenicity Data Bases Identified a 6 Angstrom Lipophilic Toxicophore Associated with Estrogenicity and Carcinogenicity



17β-estradiol



Estrogenic chemicals painted according to lipophilicity. The 6Å 2D biophore is illustrated in 4-methylphenol. All chemicals shown possess the physical distance requirements of the biophore. *Methoxychlor metabolite = 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane; 2-chlorobiphenyl metabolite = 2-chloro-4-hydroxybiphenyl.

Absence of 6Å Descriptor Among Phytoestrogens

Chemical	6Å Descriptor
Diethylstilbestrol	+
β-Estradiol	+
17α-ethinyl estradiol	+
Estrone	+
Resveratrol	+
Genistein	-
Kaempferol	-
Quercetin	-
Phloretin	-
Chrysin	-
Galangin	-
Apigenin	-
Coumestrol	-

Other phytoestrogens also all negative

Resveratrol, present in Grapes, has been proposed as a Cancer Chemopreventative Agent.

The potential toxicity of Resveratrol has not been investigated. SAR Models of Toxicological Phenomena may be a rapid and cost-effective approach.

Major Biolobical Activities of Resveratrol

Antibacterial and antifungicidal activities

Antioxidant activity

Free radical scavenging

Inhibition of lipid peroxidation

Inhibition of eicosanoid synthesis

Inhibition of platelet aggregation

Chelation of copper

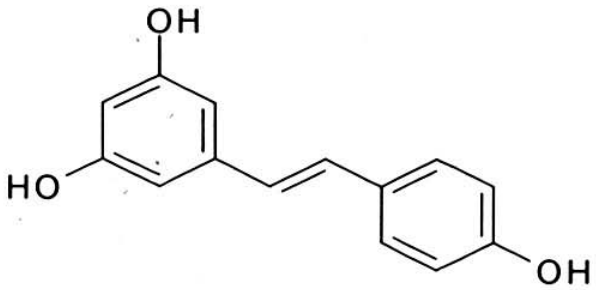
Anti-inflammatory activity

Vasorelaxing activity

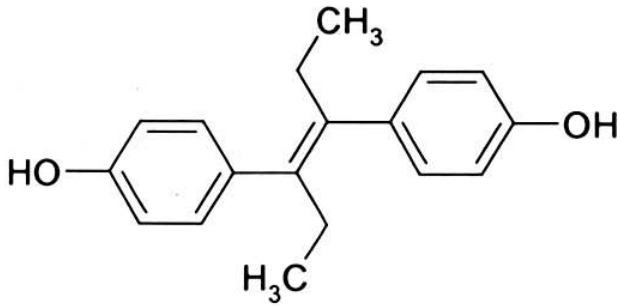
Modulation of lipid and lipoprotein metabolism

Oestrogenic/anti-oestrogenic activity

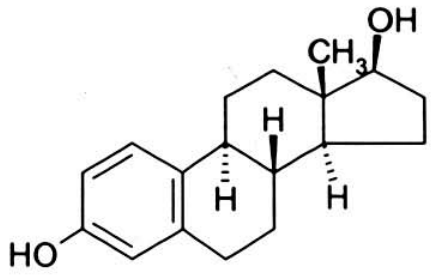
Anticancer activity



Resveratrol

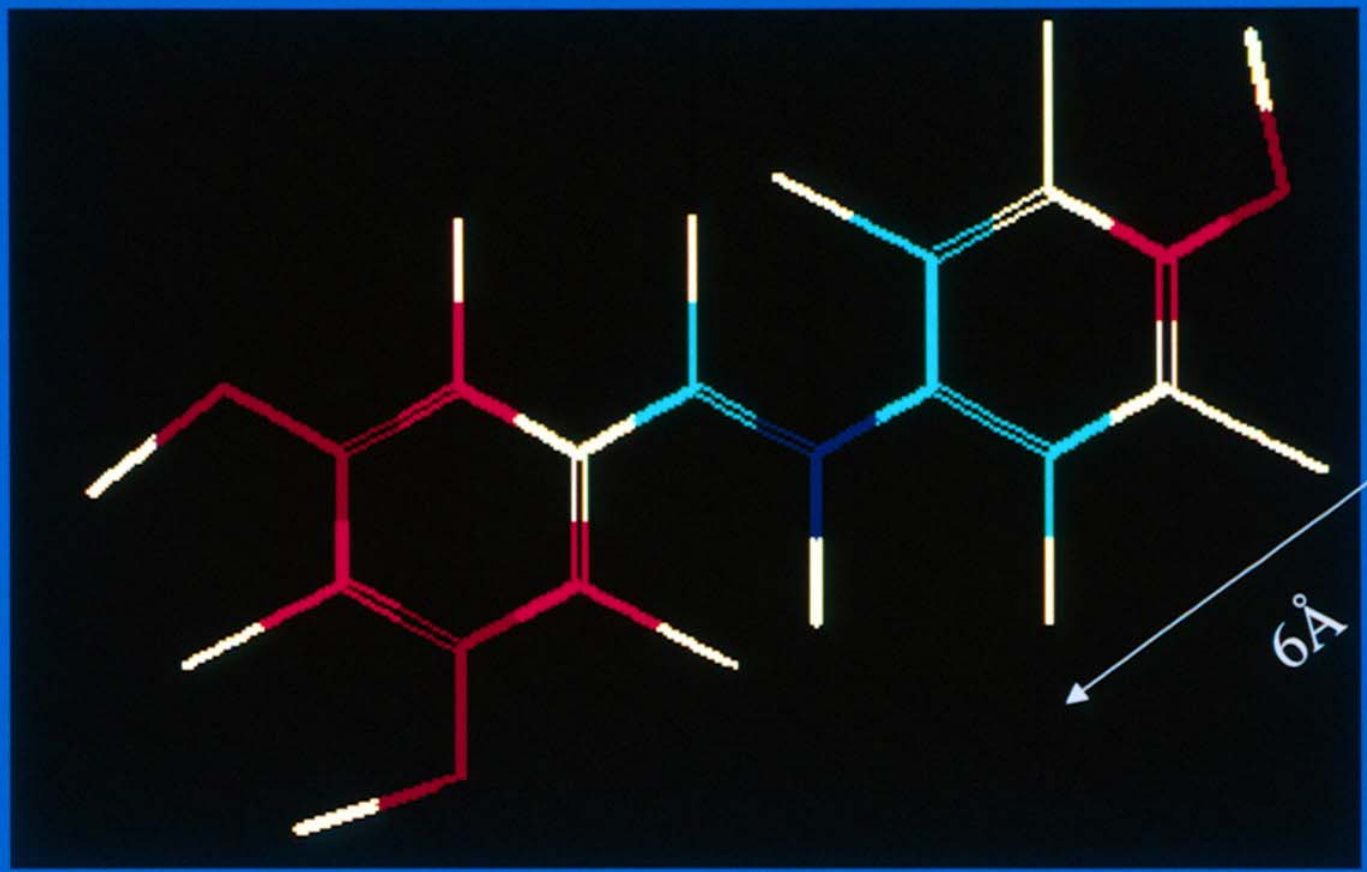


Diethylstilbestrol



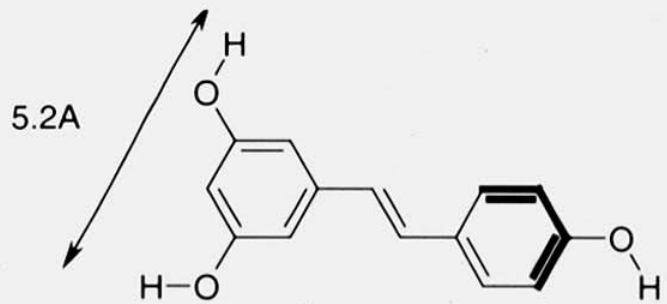
β-Estradiol

Structures containing lipophilic phenol moieties



Structure of resveratrol identifying lipophilic structures as well as the 6Å distance descriptor

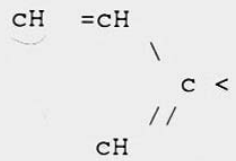
```
The molecule contains the Toxicophore   (nr.occ.= 1):
2D fragment : [OH -]  <-- 5.2A -->  [OH -]
 *** 10 out of the known  11 molecules ( 91%) containing such a
Toxicophore are  Developmental Toxicants
 *** QSAR Contribution :              Constant is            41.69
  ** The following Modulators are also present:
         Log partition coeff.= 3.52 ;LogP contribution is    -2.82
         Water  solubility   = 4.62;WS contribution is       -4.16
                                                            ------
  ** Total projected QSAR activity                          34.71

The molecule also contains the Toxicophore:

              cH  =cH
                   \
                    c <
                   //
                 cH

*** 10 out of the known 12 molecules (83%) containing such a Toxicophore
are Developmental Toxicants
*** QSAR Contribution :                    Constant is    39.00
 ** Total projected QSAR activity                         39.00
*** The probability that this molecule is a Developmental Toxicant is 94%
**
 ** The projected  potency is   74.0     CASE units **
```



Predicted Developmental Toxicity of Resveratrol

Derivation of a Structural Toxicophore Associated with Developmental Toxicity

The 12 Molecules containing the toxicophore:



N*	Chemical	Toxicity
2	Propachlor	Active
2	1-Butyl-3-sulfanylurea	Active
1	17-β-Estradiol	Active
2	Aminopterin	Active
4	Diethylstilbestrol	Active
2	Tubocuranine chloride	Marginal
2	Methotrexate	Active
4	Methoxychlor	Active
2	Methyl parathion	Inactive
2	Chlorambucil	Active
1	Linuran	Active
2	Nitrofen	Active

*N indicates the number of toxicophores in the chemical.

Derivation of a 2D Toxicophore Associated with Developmental Toxicity

The 11 Molecules containing the Toxicophore:

2D fragment formula : **[OH -] <-- 5.2A --> [OH -]**

Chemical	Toxicity
Streptomycin	Active
Toluene-3,5-diamine	Active
Aminopterin	Active
Methotrexate	Active
Dexamethasone	Active
6-Azauridine	Active
5-Fluorouracil	Active
Tetracycline	Marginal
Cytarabine	Active
Penicillamine	Active
Propylthiouracil	Active

Predicted Toxicological Profile of Resveratrol

SAR Model	Prediction
*Carcinogenicity (Rodents)	N(?)
Mutagenicity (*Salmonella*)	N
Structural Alert for DNA Reactivity	N
Mutation/Recombination (Drosophila)	N
Mutation (Mouse Lymphoma)	P
SOS Error Prone DNA Repair	N
Sister Chromatid Exchange (*in vitro*)	P
Sister Chromatic Exchange (*in vivo*)	N
Chromosomal Aberration (*in vitro*)	N
Induction of Micronuclei (*in vivo*)	N
Cell Transformation (Balb/3T3)	N
UDS Induction	N
α 2μGlobulin Nephropathy	N
Inhibition of Human Cytochrome P4502D6	N
Binding to Ah Receptor	N
Malsegregation (Yeast Aneuploidy)	N
Tubulin Polymerization Inhibition	N
Inhibition of GJIC	N
Cellular Toxicity (Balb /3T3)	P
Cellular Toxicity (Hela)	P
Developmental Toxicity (Mice)	P
Developmental Toxicity (Rats)	P
Developmental Toxicity (Rabbits)	N
Developmental Toxicity (Hamsters)	N
Developmental Toxicity (Humans)	N
Anticarcinogenesis (Rodents)	N

CONCLUSION

Resveratrol has too many Liabilities to be used as a Dietary Supplement for Prolonged Periods especially as its Beneficial Effects in Humans have, as yet, not been established.

Indole-3-Carbinol

SAR-Generated Profile Indicates Potentials:

1.to Act as a Rodent Anticarcinogen.

2.to Inhibit Human Cyp2D6 (This is not necessarily a Liability, as many Therapeutics, such as β -Blockers, also inhibit Cyp2D6.)

Indole-3-carbinol

The molecule contains the Biophore

```
      c. =cH
           \
    c.       cH
     \\     //
      cH  -cH
```

*****12 out of the known 14 molecules (86%) containing such a biophore are inhibitors of P4502D6.**

*****QSAR Contribution : Constant is -25.48**

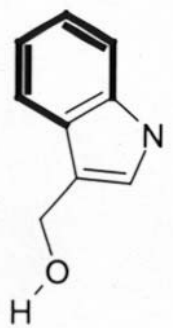
**** The following Modulators are also present:**

Ln Nr.Bi/Mol.Wt. = -4.99 ; Nr.Bioph/MW contribution is	**71.27**
Hard/Soft index is = 0.67 ; Its contribution is	**-12.08**

**** Total projected QSAR activity**	**33.71**

***** The probability that this molecule is an Inhibitor of P4502D6 is 86.0% ****

**** The projected P450 inhibitions activity is 33.7 CASE units ****

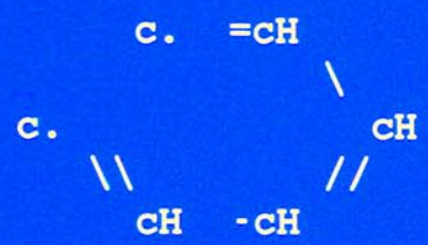


The ability of indole-3-carbinol to inhibit human cytochrome P4502D6

Cytochrome P450 2D6 Inhibition

Indole-3-carbinol

The 14 Molecules containing fragment :



are :

1	in molecule	4	Propranolol	of activity	38
1	in molecule	11	Cinchonine	of activity	43
1	in molecule	12	Ajmalicine	of activity	97
1	in molecule	14	Gelsemine	of activity	14
1	in molecule	53	Ajmaline	of activity	52
2	in molecule	59	Amitriptyline	of activity	30
1	in molecule	60	CHLORPROMAZINE	of activity	43
1	in molecule	61	Domperidone	of activity	42
1	in molecule	62	Yohimbine	of activity	28
1	in molecule	66	Fluphenazine	of activity	57
2	in molecule	83	Carbamazepine	of activity	14
1	in molecule	89	Debrisoquin	of activity	28
1	in molecule	90	STRYCHNINE	of activity	26
2	in molecule	93	Nortriptyline	of activity	38

BPThampatty [2001]

The molecule contains the Biophore OH -CH2

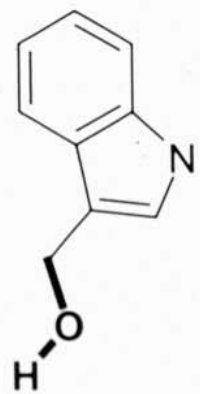
*** 5 out of the known 6 molecules (83%) containing such a Biophore are Anticarcinogens.**

***** QSAR Contribution : Constant is 39.00**

**** Total projected QSAR activity 39.00**

***** The probability that this molecule is an Anticarcinogen is 86.0% ****

**** The projected anticarcinogenic potency is 39.0 CASE units ****

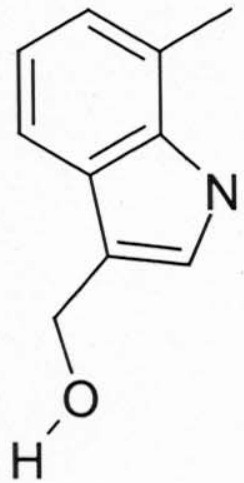


Projected anticarcinogenic activity of indole-3-carbinol

Should we wish to Abolish the Potential to Inhibit Cyp2D6, we can Methylate the C-7 Position?

1. Abolishes the Cyp2D6 Inhibitory Activity
2. Does not create Any New Toxicological Liabilities.
3. Augments the Anticarcinogenic Potential by generating an additional Pharmacophore

** The molecule does not contain any known Biophore **
it is therefore presumed to be INACTIVE



Prediction of the inability of 7-methyl indole-3-carbinol to inhibit human cytodrome P4502D6.

The molecule contains the expanded Biophore (nr.occ.= 1):

```
cH  =cH
     \
      c
     //
   c.
```

*** 6 out of the known 6 molecules (100%) containing such a Biophore are anticarcinogens. (conf.level= 98%)

*** QSAR Contribution : Constant is 39.00

** Total projected QSAR activity 39.00

The molecule also contains the Biophore:

OH -CH2-

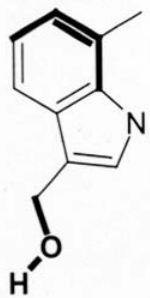
*** 5 out of the known 6 molecules (83%) containing such a Biophore are anticarcinogens. (conf.level= 94%)

*** QSAR Contribution : Constant is 39.00

** Total projected QSAR activity 39.00

** The probability that this molecule is an anticarcinogen is 90.0% **

** The projected anticarcinogenic potency is 78.0 CASE units **



The projected anticarcinogenic activity of 7-methyl indole-3-carbinol

γ-Butyrolactone

Dietary Supplement
Illicit Recreational Drug
Animal Toxicity: Negative
Humans: Coma, Deaths

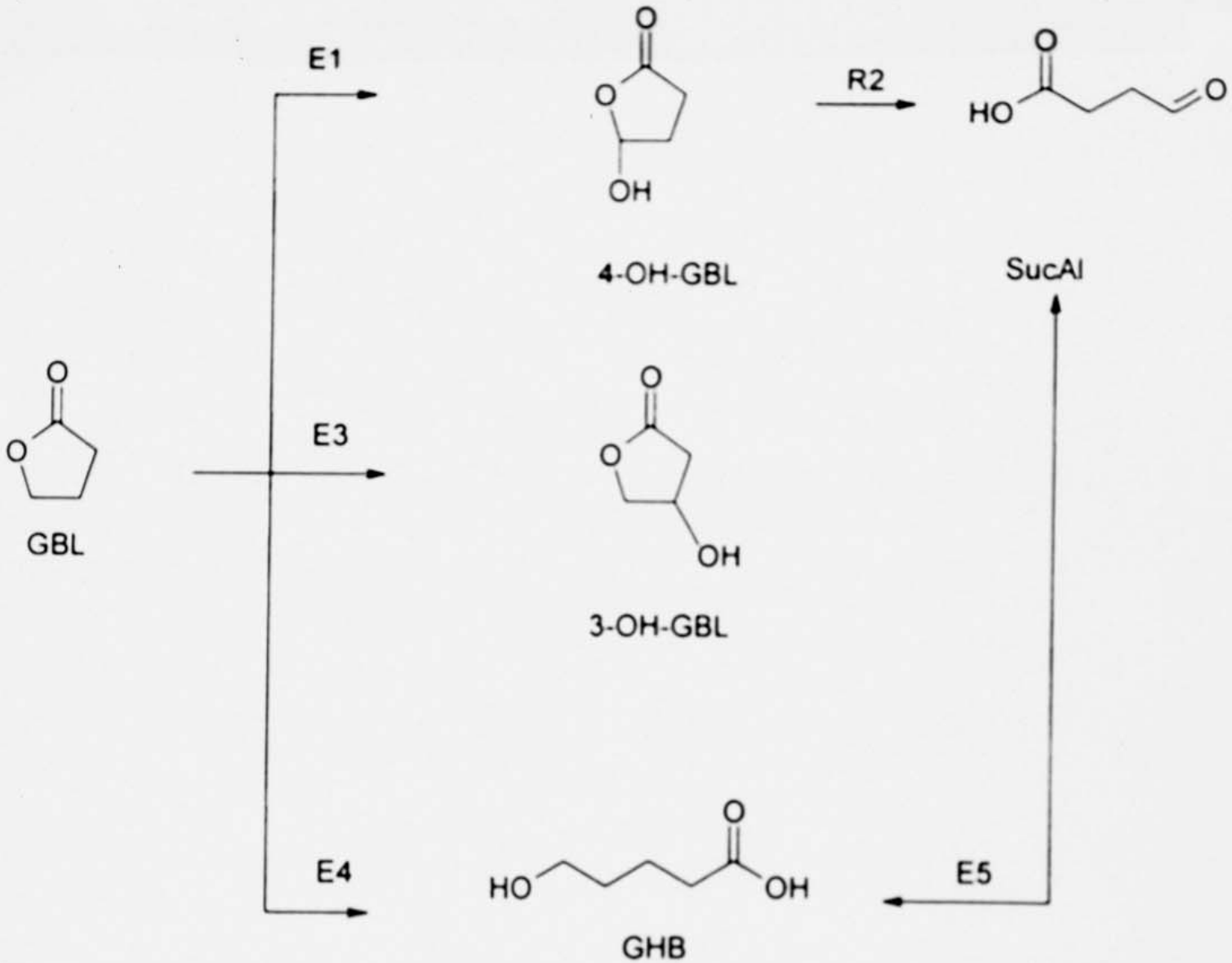


FIGURE 1 Putative biotransformations of γ-butyrolactone. E1 and E3 are P-450 monoxygenase catalyzed hydroxylations; E4 represents an esterase-catalyzed hydrolysis (i.e. gamma-lactonase); E5 is catalyzed by GHB dehydrogenase. R2 represents a spontaneous decomposition.

γ-Butyrolactone (GBL)

Hypothesis:

CYP2D6 inhibition not toxic per se
Humans: Mixed exposures
GBL users also abuse other Agents, ethanol
Inhibition of CYP2D6 may inhibit detoxification of other drugs!

Identification of Candidate Agents

Data Mining:

Virtual Similarity Index

Identification of new candidates

Similarity Indices: Procedure

1. Generate "Virtual" Toxicological Profiles of Test Chemicals
2. Compare Nature of Overlaps
3. Determine expected Prevalence of Such Overlaps among 10,000 Chemicals in Commerce and Industry

Virtual Toxicological Profiles

Model	Genistein	Curcumin	Rofecovib
CACombined	act	I	I
SalmNTP	act	I	I
MLA/GT	act	act	act
SensIr	act	act	I
ACD	act	act	I
RespHyper	I	I	act
EyeIrr	I	act	I
MoMTD	I	I	I
RaMTD	act	act	I
BalbTox	act	act	act
Minnow	act	act	I
Rat LD50	act	I	I
HamDev	I	I	I
Hum/FDA De	I	I	I
SCEinvitro	act	act	act
MoSCE	act	act	act
ChrAber	I	act	I
Micronucle	act	act	act
UDS	I	I	I
SOS Chromo	I	I	I
alpha2mu N	I	act	I
Ah recepto	I	I	I
HeLaTox	act	act	I
Biodegrada	act	act	I
iGJIC	I	I	I
Skin Perme	act	act	act
inhP4502D6	I	I	I

*Only a subset of SAR models were used

Similarity Indices

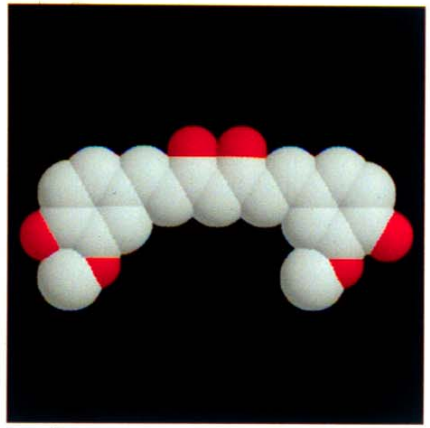
Genistein + Curcumin	0.6%
Genistein + Curcumin + Resveratrol	0.6%*
Genistein + Rofecoxib	5.6%
Curcumin + Rofecoxib	5.4%
Resveratrol + DES	0.8%
Resveratrol + Rofecoxib	6.6%
DES + Rofecoxib	5.6%

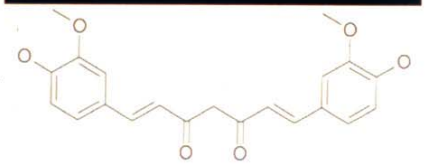
Based upon toxicological profiles of 10,000 chemicals

* Probability that this is a result of chance is 0.2%

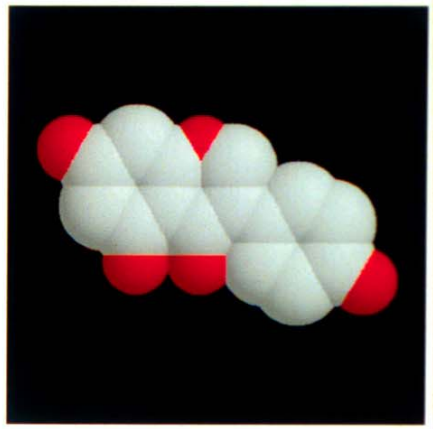
Chemicals Sharing Profile with Genistein and Curcumin

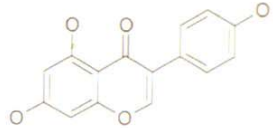
5-Hydroxytienilic Acid
Dobutamine
Ethacrynic Acid
Flecainide
LY171883
Metabutoxycaine
Propanidid
Protriptyline
Tienilic Acid
Tienilic Acid (Isomer3)
4'-Hydroxyflavone
4',5-Dihydroxyflavone
4',7,8-Trihydroxyflavone
3',5,7-Trihydroxy-4'-Methoxyflavone
Benzenesulfonamide, N-(((1-met
2-Propanol, 1-((1,1-dimethylet
Spiro(benzofuran-2(3H), 1'-(2)
Spiro(benzofuran-2(3H), 1'-(3)



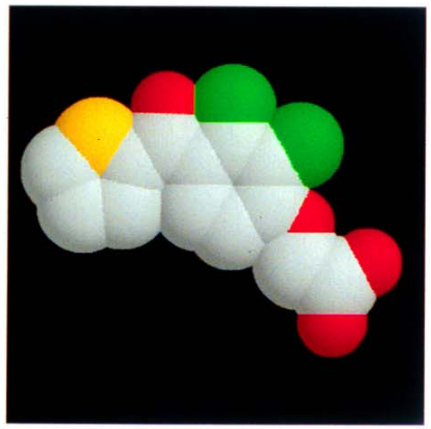


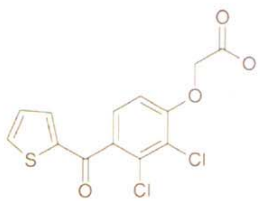
Curcumin



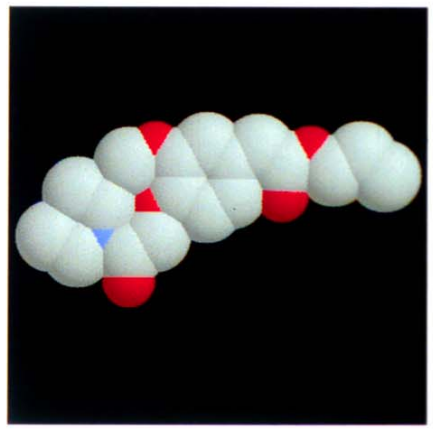


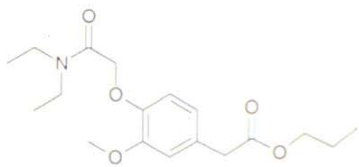
Genistein





Tienilic Acid





Propanamid

Future

Identification of Targets → Models

Pharmacogenomics

(1) Readily integrated into other computer-based models

(2) To define new targets → models

(3) To explain variation in individual response → reduce uncertainty