

Identification of PPAR γ agonists from natural products



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Outline

- Metabolic syndrome and Type 2 Diabetes
- PPARs
- PPAR agonists from natural sources
- Methods for identification of PPAR agonists
- Examples
 - *Echinacea purpurea*
 - *Salvia officinalis*
 - *Sambucus nigra*
 - *Pistacia lentiscus* var. *Chia*
- Conclusions

Fedmeepidemi sætter ny rekord

Nu lider 600.000 voksne danskere af svær overvægt. Sundhedsminister Bertel Haarder er »fortvivlet« over fedme.

AF **ANDERS LEGARTH SCHMIDT**

Et rekordhøjt antal voksne danskere er nu svært overvægtige og har dermed stærkt forhøjet risiko for at få kroniske sygdomme og dø for tidligt.

600.000 borgere over 15 år vejer så meget, at de lider af fedme. Det viser en beregning, Politiken har foretaget på baggrund af tal om udbredelsen af svær overvægt fra Den Nationale Sundhedsprofil koblet

*Fedmekurven knækker, men ikke af sig selv
Vi tror på forskere, der giver grund til forsigtig optimisme, skriver
Haarder og Høegh.*

Du glemmer fedmeforskningen, minister

Ekspertter anklager Haarder og Høegh for skønmaleri om fedme

Overvægtsforening: Sådan knækker vi fedmekurven

POLITIKEN.DK

Sukkersyge rammer oftest singler

Bor du alene, har du større risiko for at få den skadelige diabetes.

AF **METTE GULDAGGER**

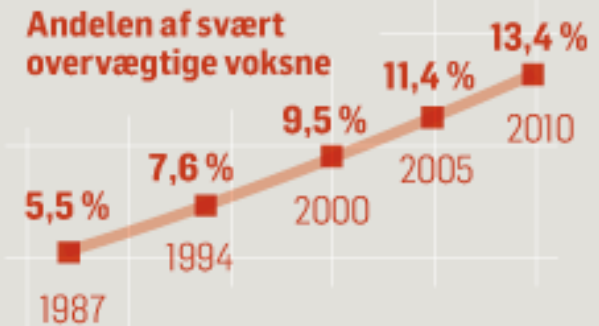
Over en million danskere har ikke en samlever, som de kan rask gåtur. De bor alene og er dermed del af en gruppe, der lider af folkesygdom diabetes 2, end gifte folk gør.

Gift dig eller bliv syg

... som trækker dem ud på en
... mere af den hastigt voksende

Flere svært overvægtige

Andelen af svært overvægtige* voksne danskere er mere end fordoblet siden 1980'erne. Nu lider over hver ottende af fedme.



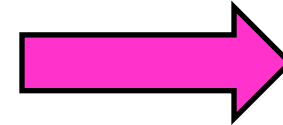
*For at være svært overvægtig, skal man have et Body Mass Index (BMI) over 30. Det svarer til, at en person på 182 centimeter vejer 100 kilo.

Kilder: Forebyggelseskommissionen og Sundhedsstyrelsen - CN - 11279

Metabolic diseases

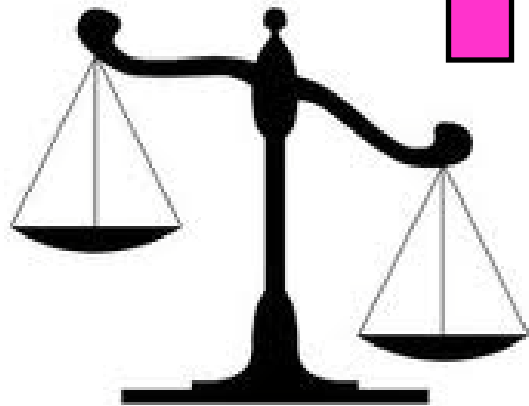
METABOLIC SYNDROME

Dyslipidaemia
Hypertension
Hypercoagulation
Pro-inflammatory state
Pro-thrombotic state
Insulin resistance

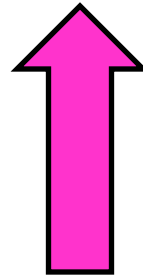


Atherosclerosis
Cancer
Hypertension
Alzheimers
Type 2 diabetes
CVDs
Etc.....

Energy intake



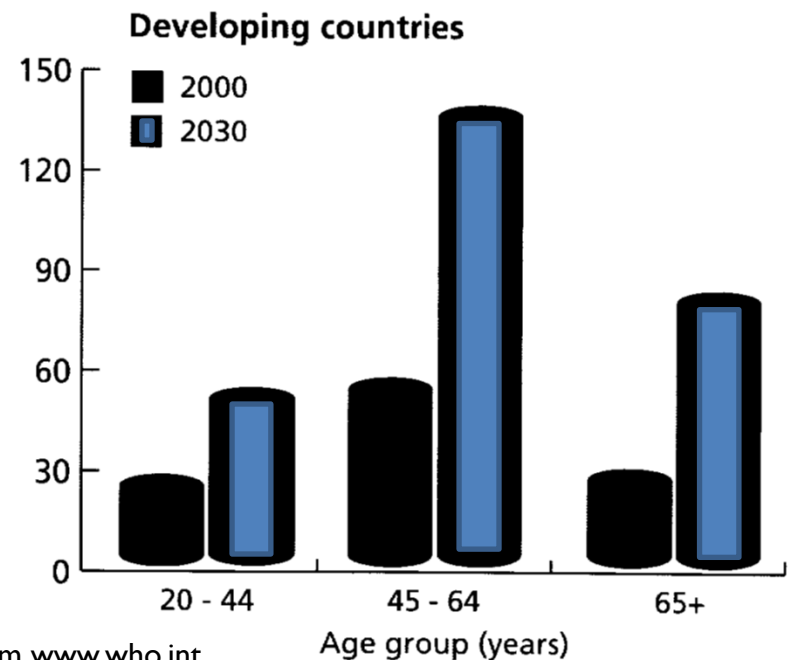
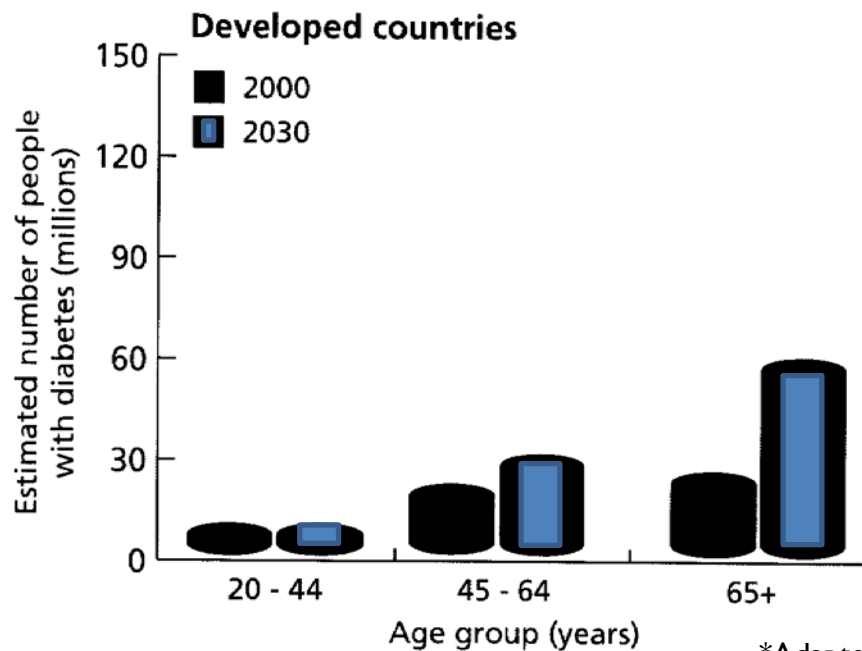
Energy expenditure



Prevalence of diabetes

In Denmark:

- 270.000 people have been diagnosed with diabetes (x2 within 10 years!!)
- 10 % have type 1, 80 % have type 2, and the rest other forms
- The Danish Diabetes Society estimates that 750.000 people have pre-diabetes and that ca. 250.000 people have undiscovered T2D

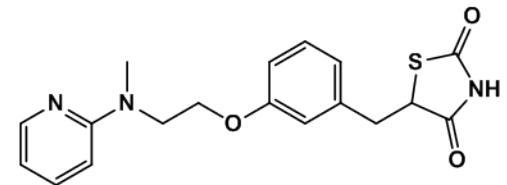


*Adapted from www.who.int

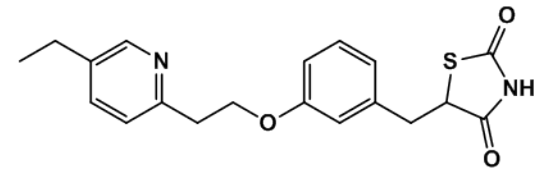
Therapies of T2D

- Initial therapies
 - Life style changes** – to decrease weight and increase activity
 - Metformin** – to decrease hepatic glucose production and thereby reduce fasting plasma glucose
- Additional therapies
 - Sulfonylureas** – to increase insulin secretion from pancreatic β -cells. Major side-effect is hypoglycaemia
 - Thiazolidinediones (TZDs)** – to reduce insulin resistance. Side-effects are weight-gain, oedemas etc.
 - Others** – bile acid sequestrants, meglitinides, α -glucosidase inhibitors etc.

TZDs function as insulin sensitizing drugs by binding to and activating the nuclear receptor PPAR γ



Rosiglitazone (TZD)

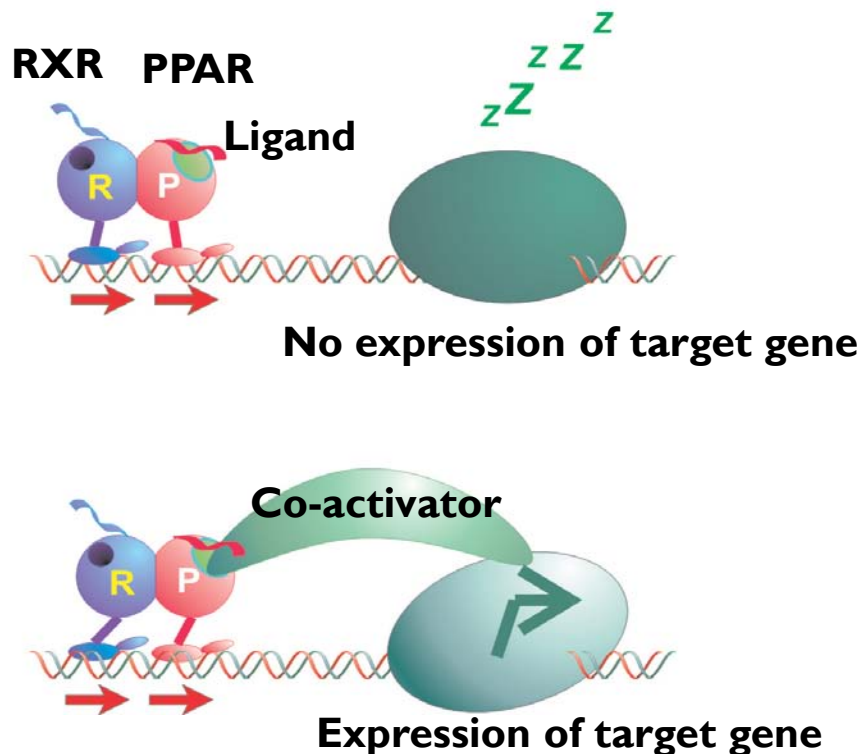


Pioglitazone (TZD)

Nuclear receptors

Nuclear receptors are ligand-dependent transcription factors that upon recruitment of a certain set of co-activators have significant influence on a large number of physiological processes.

Mechanism of action:



- **Nuclear receptors are still promising targets for metabolic diseases**
- **In particular, the receptors that function as "lipid sensors" e.g.**
 - **PPAR**
 - **LXR**
 - **FXR**

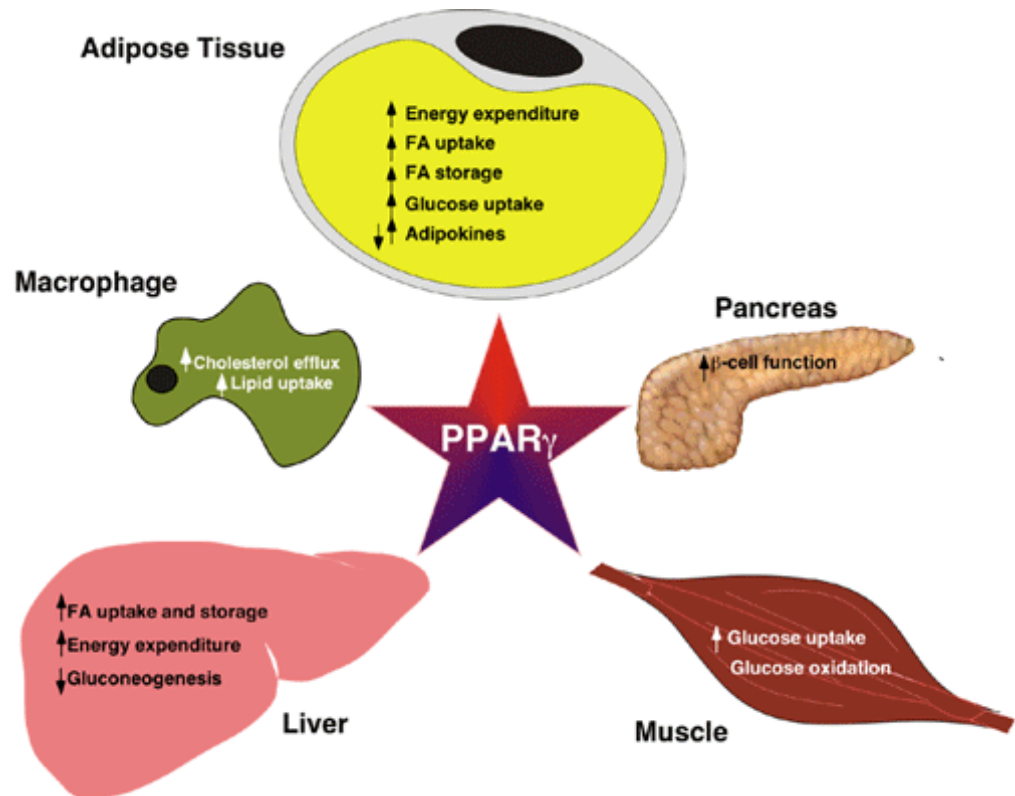
PPARs

- PPAR = Peroxisome Proliferator-Activated Receptor
- Three subtypes exist: PPAR α , PPAR δ , and PPAR γ
- All PPARs are highly involved in the regulation of lipid and glucose metabolism
- Endogenous ligands for PPARs are fatty acids, eicosanoids, prostaglandins etc.

PPAR γ is a key regulator of adipocyte differentiation.

Activation of PPAR γ =>

”a redistribution of fat from visceral to subcutaneous adipose tissue”



Auwerx et al. (2003) Nuclear Receptor Signalling I, e006

Partial PPAR γ agonists/Selective modulators

- Criteria for a better safety profile:
 - High selectivity
 - Full insulin sensitizing effect
 - No adipogenic activity
 - No or reduced fluid retention
 - No liver toxicity
 - No heart enlargement
- Partial PPAR γ agonists are ligands that are able to activate the receptor but not as well as a full agonist
- Evidence suggests that partial PPAR γ agonists cause less fat deposition, plasma volume expansion, and hepatotoxicity

Natural products are promising candidates as partial PPAR γ agonists and modulators of other nuclear receptors

Natural products as modulators of PPAR



Herbs (here *Origanum vulgare*)



Bushes (here *Dodonea viscosa*)



Barks (here *Populus balsamifera*)



Vegetable oils (here palm oil)



Fruits (here *Mormordica charantia*)



Seeds and seed caps (here *Fraxinus excelsion*)

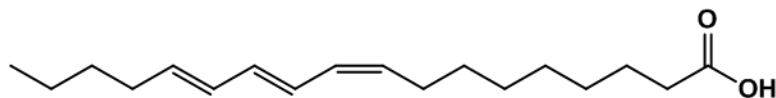


Soft corals (here *Sinularia numerosa*)



Roots (here *Rhaponticum uniflorum*)

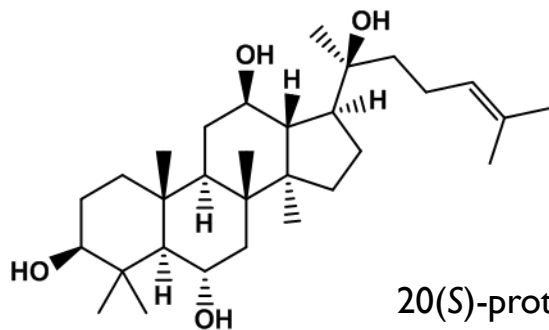
• PPAR modulators (FAs and FA-like compounds)



9Z,11E,13E conj. linolenic acid
PPAR α agonist from bitter melon

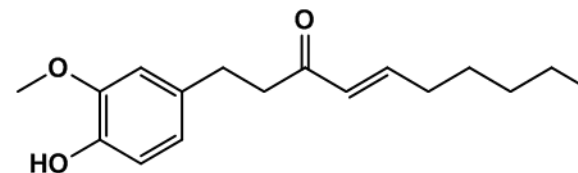
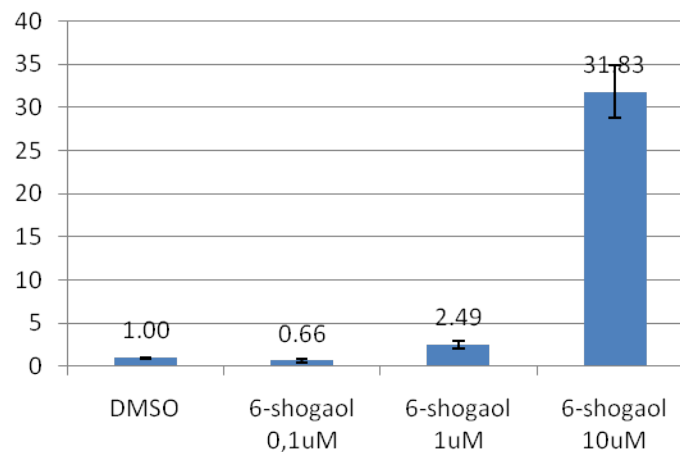
Chuang et al. (2006) *J. Biomed. Sci.* 13, 763-772

• PPAR modulators (Terpenoids)



20(S)-protopanaxatriol
PPAR γ activator from ginseng

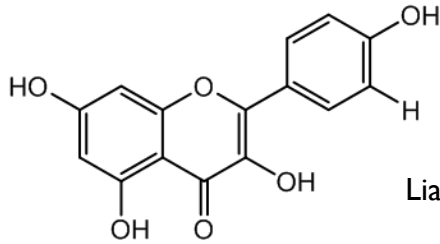
Han et al. (2006) *Biol. Pharm. Bull.* 29, 110-113



6-shogaol
PPAR γ agonist from ginger

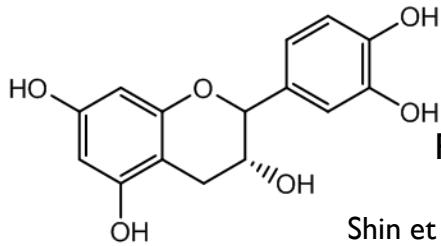
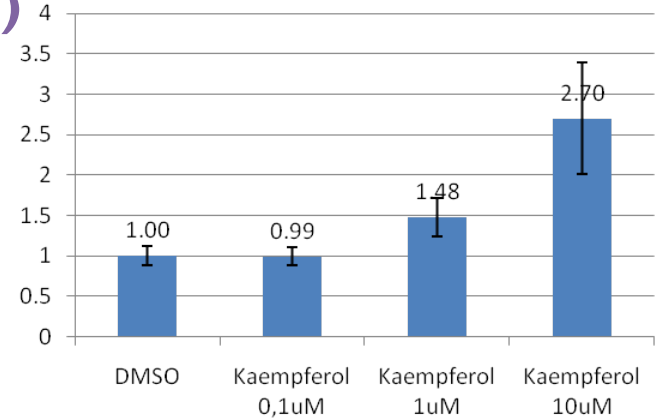
Isa et al. (2008) *Biochem. Biophys. Res. Comm.* 373, 429-434

• **PPAR modulators (Flavonoid derivatives)**



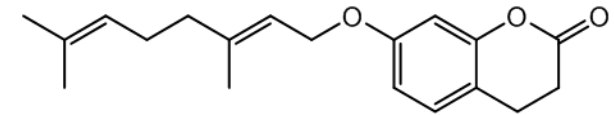
Kaempferol
PPAR γ activator

Liang et al. (2001) *FEBS Lett.* 496, 12-18



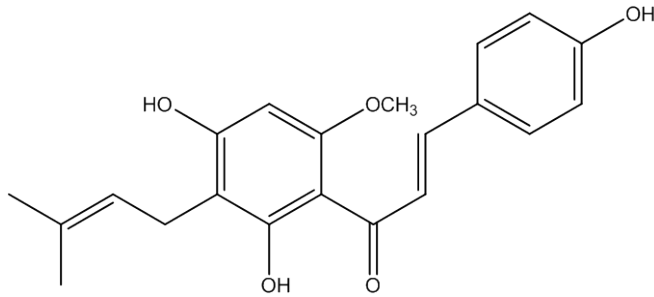
(-)-Catechin
PPAR γ activator from green tea

Shin et al. (2009) *Biochem. Pharmacol.* 77, 125-133



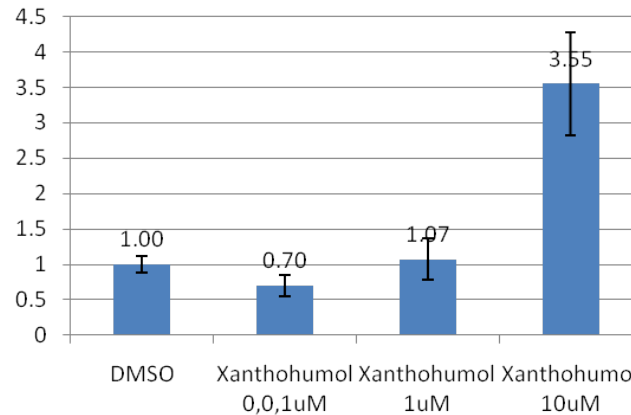
Auraptene
PPAR α and γ activator from *Citrus* sp.

Kuroyanagi et al. (2008) *Biochem. Biophys. Res. Comm.* 366, 219-225

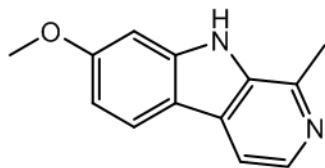


Xanthohumol
PPAR γ activator from hops

Mendes et al. (2008) *J. Biomed. Sci.* 13, 763-772



• PPAR modulators (Alkaloids)



Harmine

A well-studied PPAR γ modulator. Not a true agonist but has *in vivo* effects comparable to one although it does not affect body weight and hepatic lipid accumulation.

Waki et al. (2007) *Cell Metab.* 5, 357-370

Berberine

PPAR γ antagonist activity

Lee et al. (2006b) *Diabetes* 55, 2256-2264

• PPAR modulators (polyhalogenated compounds)

Drosophilin A and chlorophellin A

PPAR γ activators from the medicinal fungus *Phellinus ribis*

Lee et al. (2008a) *Bioorg. Med. Chem. Lett.* 18, 4566-4568

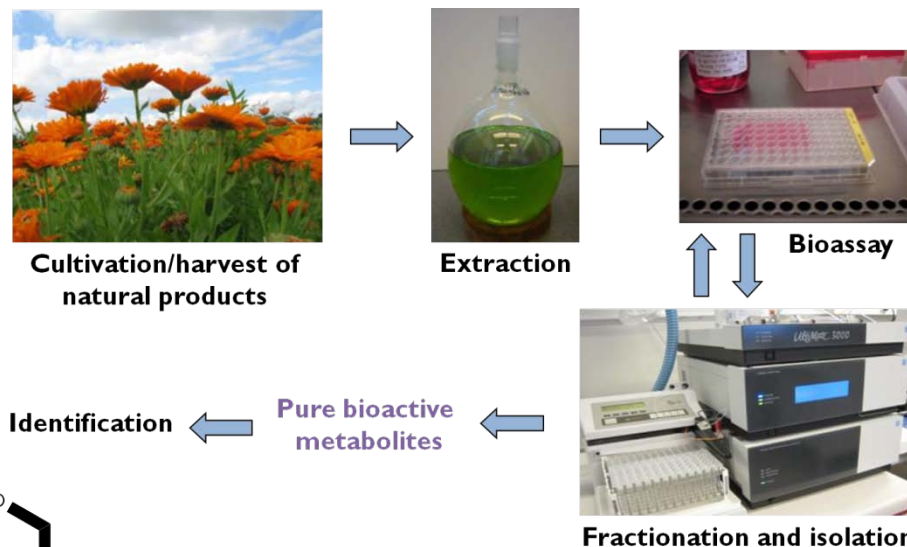
E,E-psammaplin

PPAR γ activator isolated from various marine sponges

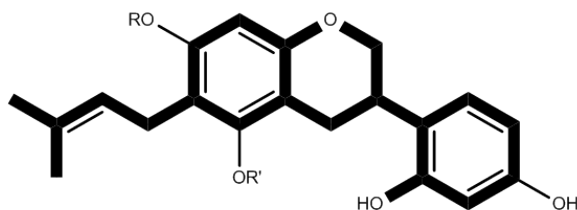
Mora et al. (2006) *J. Nat. Prod.* 69, 547-552

Ways to identify PPAR modulators

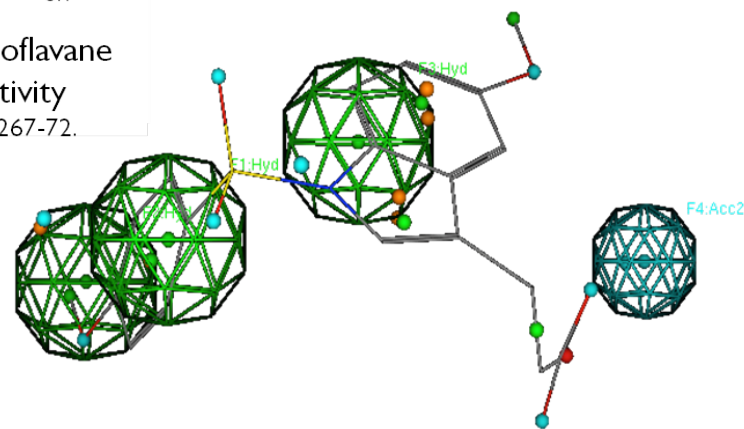
- Bioassay-guided chromatographic fractionations



- Structure-Activity

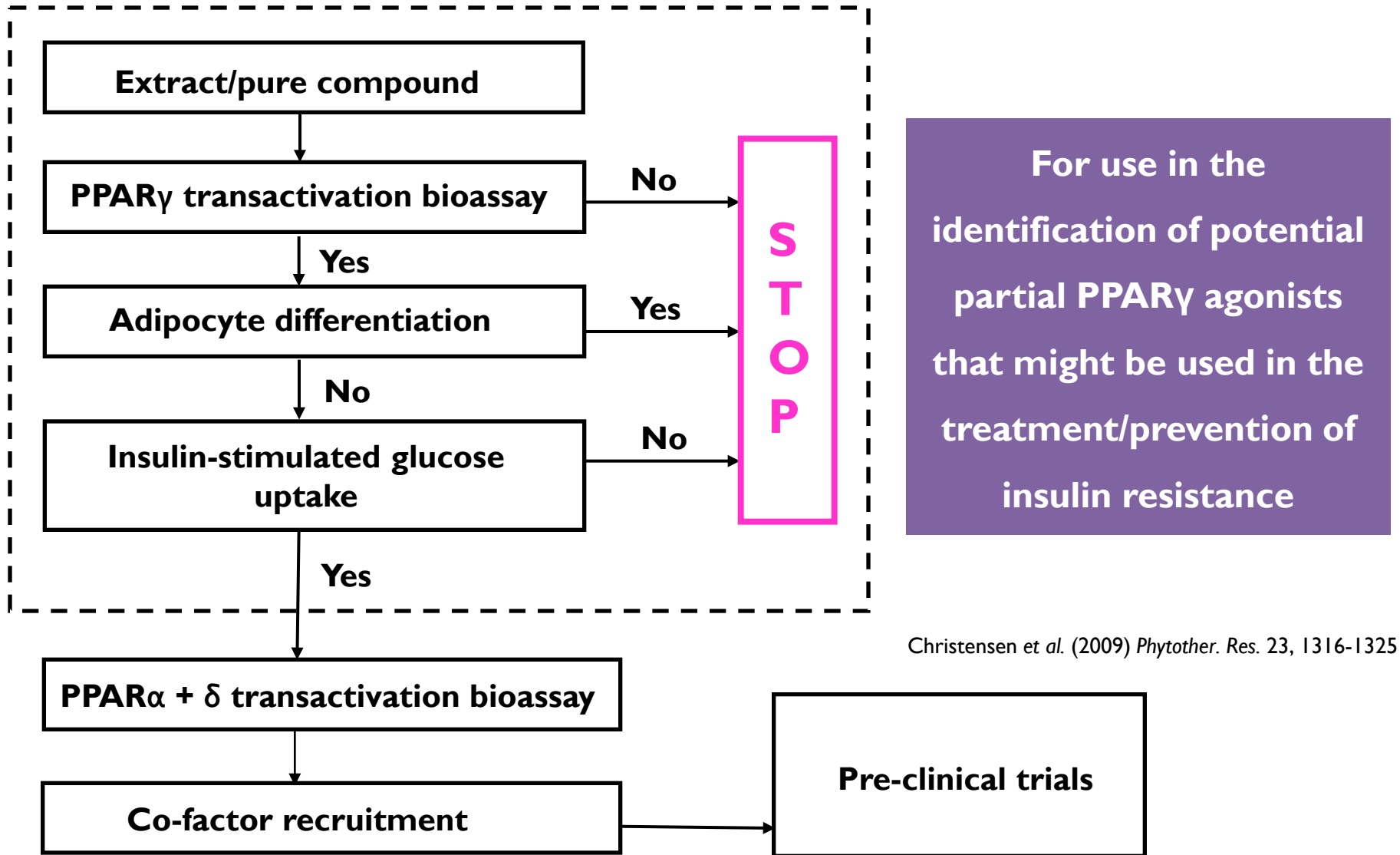


Proposed structural requirements for isoflavane skeleton for PPAR γ ligand binding activity
(Kuroda et al. (2003) *Bioorg. Med. Chem. Lett.* 13, 4267-72.)



- In silico* screening

Screening platform



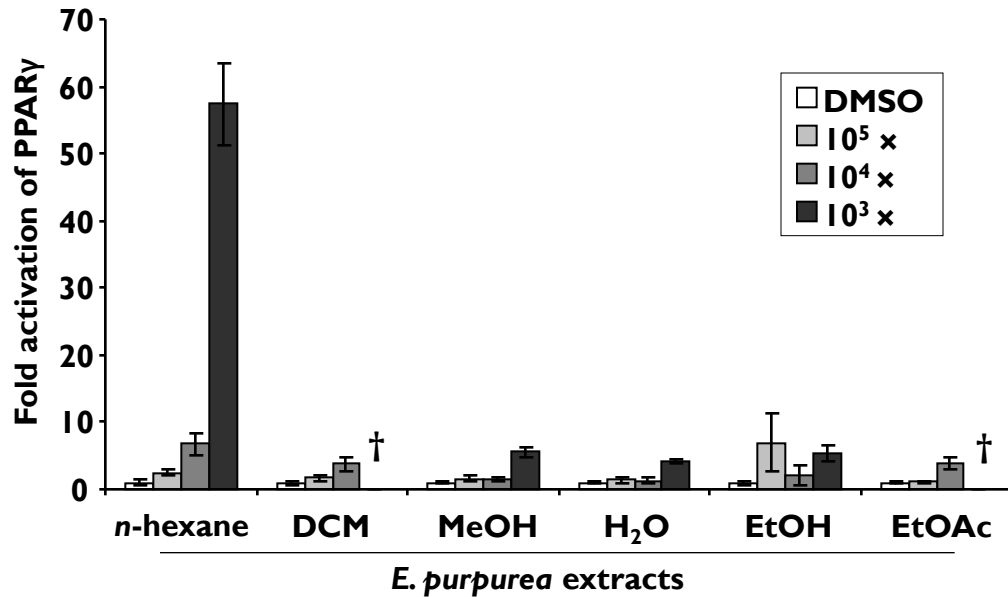
Plant species	Extract	PPAR transactivation	Ad. diff.	Glucose uptake
<i>Echinacea pallida</i> (roots)	Hex; DCM; MeOH	γ; γ; γ	tox; +; -	-; (+); +
<i>E. purpurea</i> (florets)	Hex	γ	-	-
<i>E. purpurea</i> (roots)	Hex; DCM; MeOH	γα; γα; γ	(+); -; -	-; (+); (+)
<i>Salvia officinalis</i>	DCM; MeOH; EtOAc	γ; γ; γ	-	+
<i>Sambucus nigra</i>	Hex; DCM; MeOH; EtOAc	γαδ; γα; γαδ; γδ	-	-; +; -; -

Christensen et al. (2009) *Phytother. Res.* 23, 1316-1325

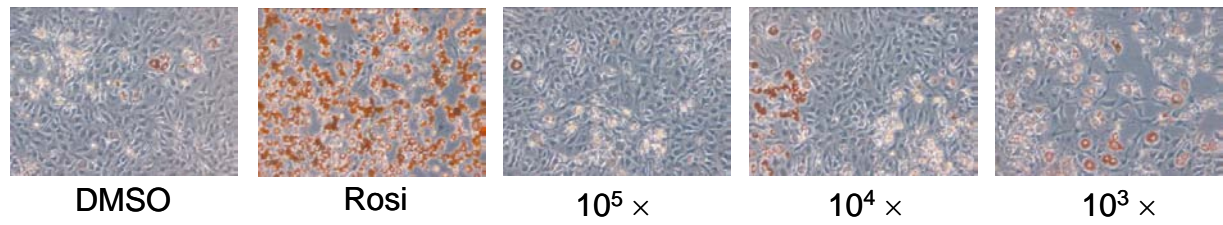
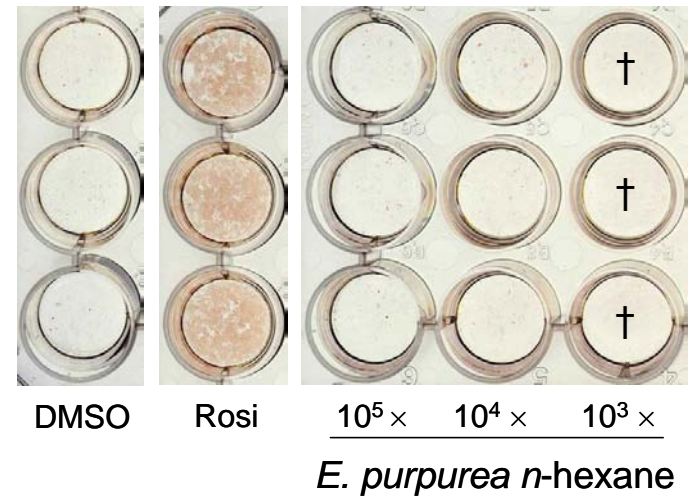
Echinacea purpurea (aerial parts)



Activation of PPAR γ



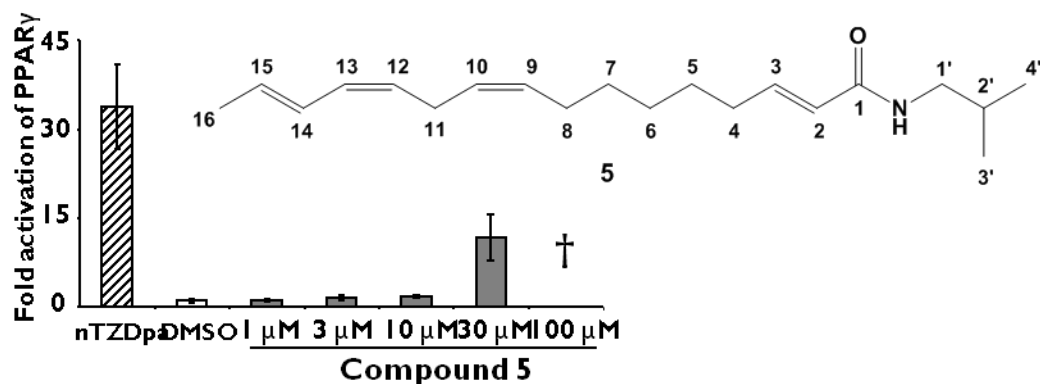
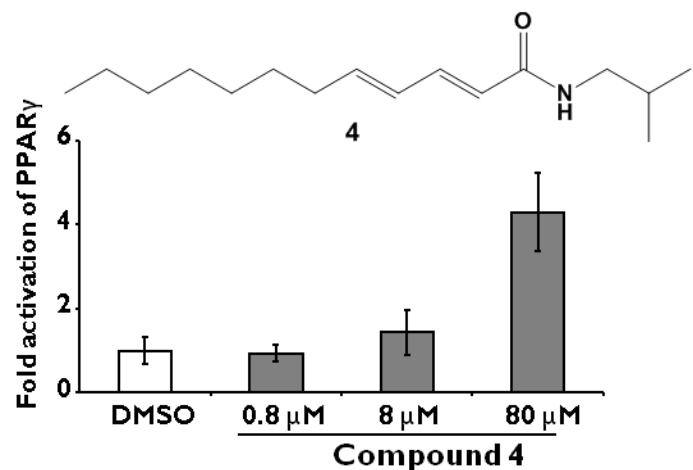
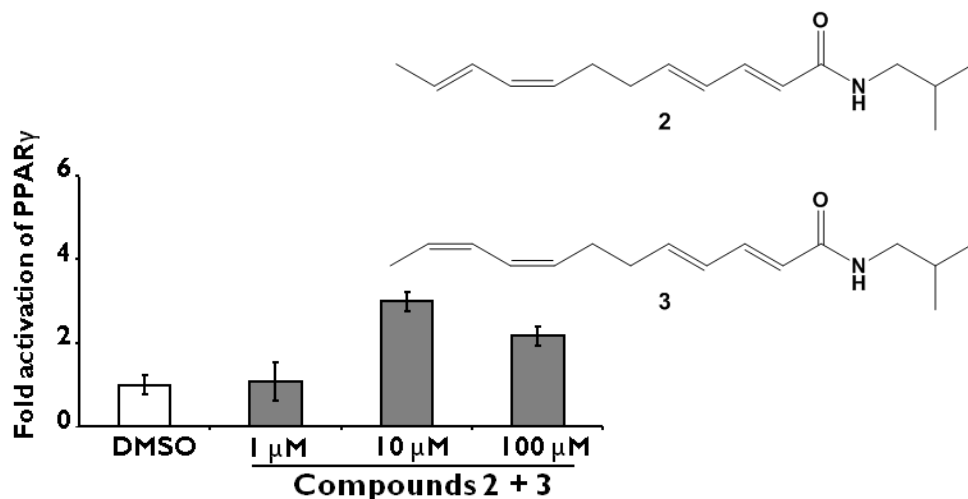
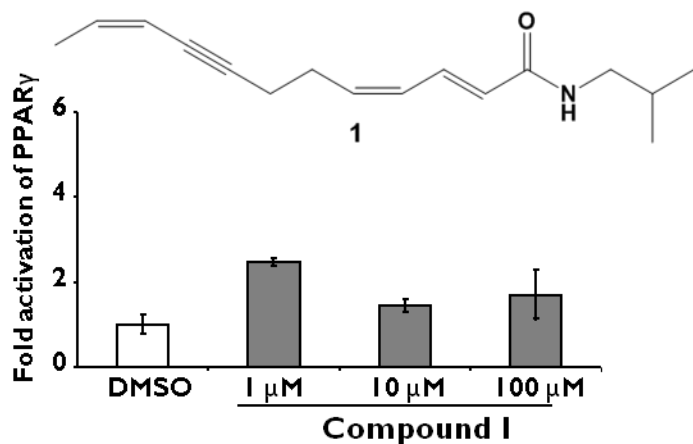
Effect on adipocyte differentiation



Christensen et al. (2009) J. Nat. Prod. 72, 933-937

E. purpurea n-hexane

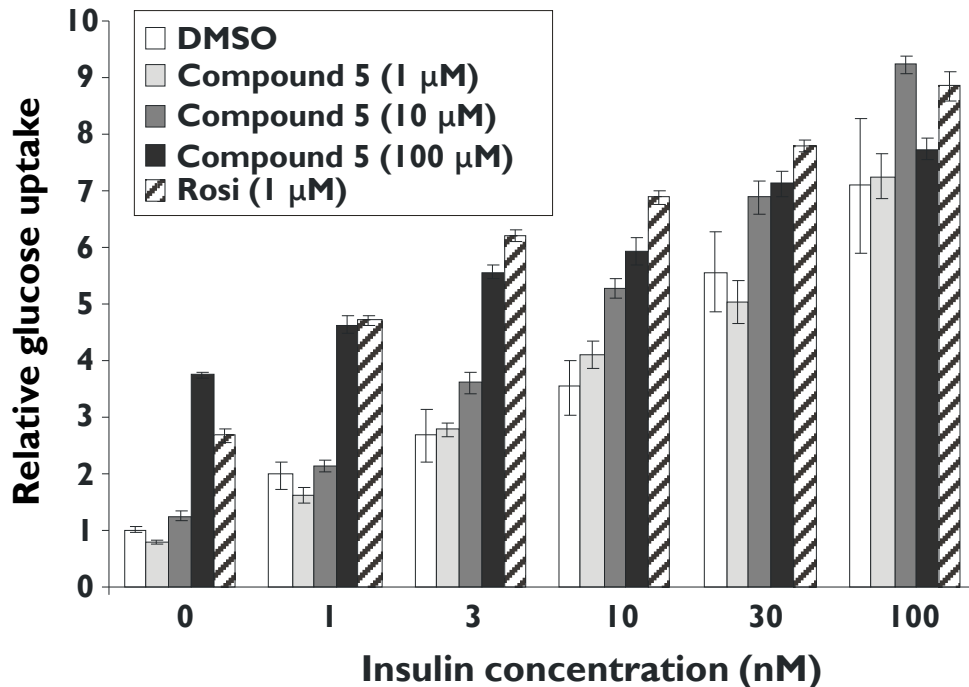
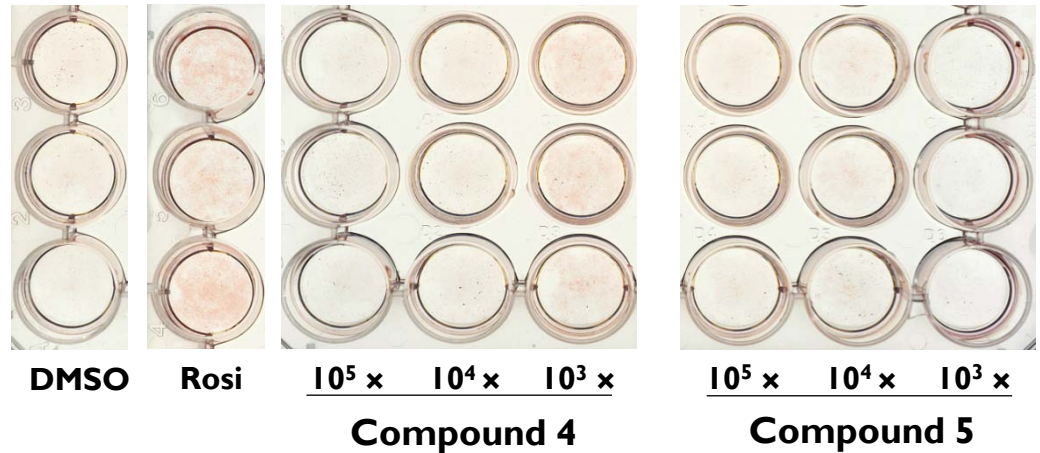
Isolation of PPAR γ -activating alkamides



Length of the carbon chain seems to be important for the degree of activation of PPAR γ by alkamides. This corresponds to data for fatty acids as PPAR γ agonists and ginkgolic acids as FXR activators

Effect on adiposity and glucose uptake

Effect on adipocyte differentiation



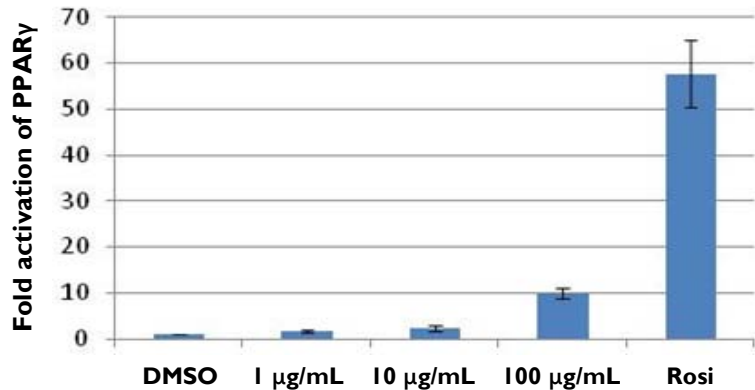
Effect on insulin-stimulated glucose uptake

Christensen et al. (2009) *J. Nat. Prod.* 72, 933-37

E. purpurea root extracts



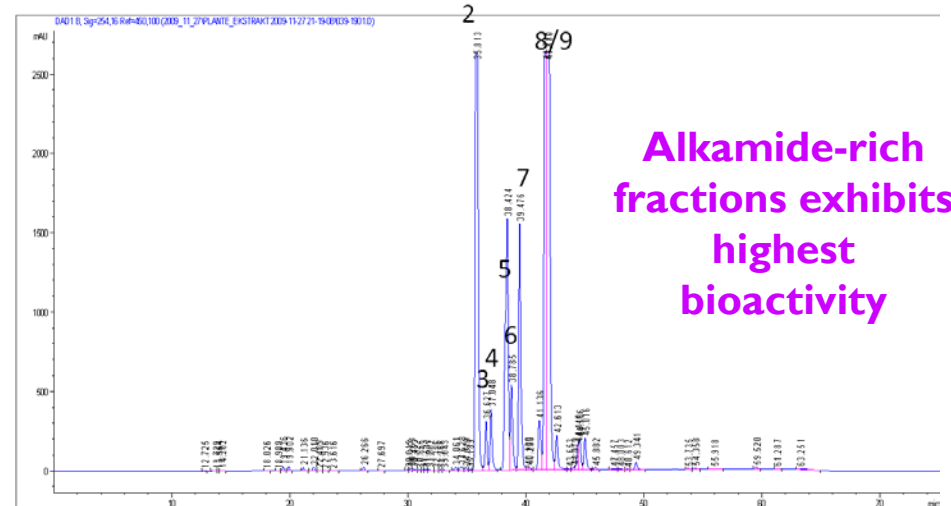
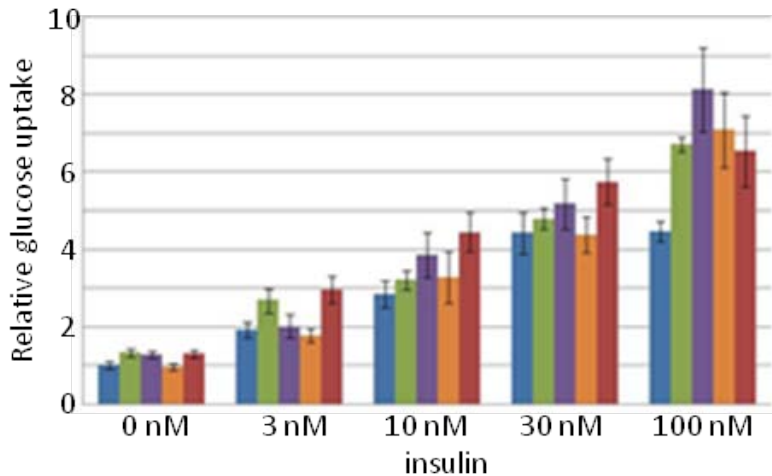
Activation of PPAR γ



Effect on adipocyte differentiation



Effect on glucose uptake in adipocytes



El-Houri et al. (2011) NNPC Oslo

Echinacea purpurea

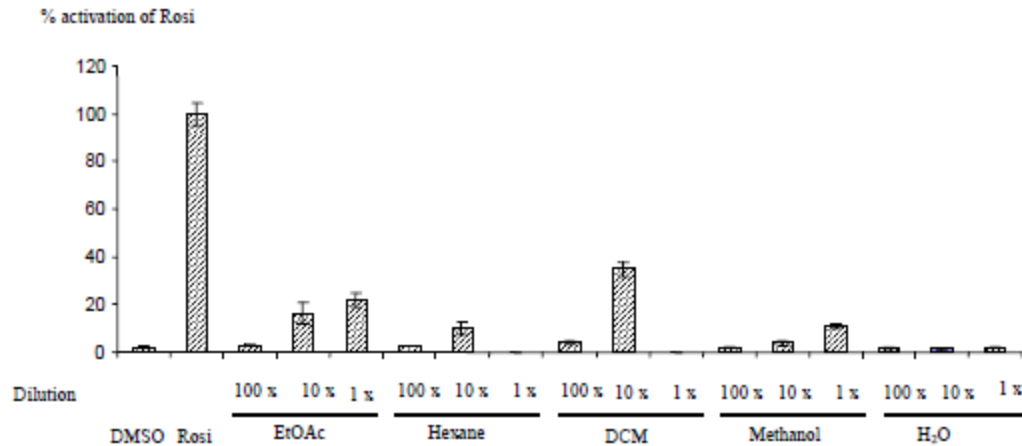


- Alkamides from roots and flowers are potential weak to moderate activators of PPAR γ with no stimulation of adipocyte differentiation
- Increases glucose uptake in adipocytes as well as in porcine muscle cells
- Length of the carbon chain seem to be important regarding PPAR γ as C16-C20 have higher activity

Salvia officinalis



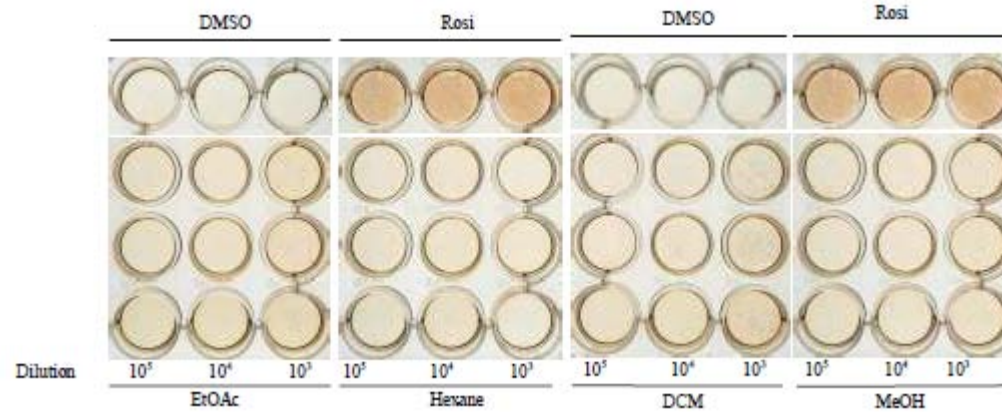
Results from general screening for potential partial PPAR γ agonists



- **Activation of PPAR by DCM, MeOH and EtOAc extracts**

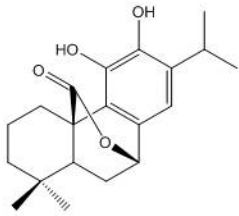
- **No stimulation of adipocyte differentiation**

- **Positive effect on insulin-stimulated glucose-uptake in adipocytes (data not shown)**

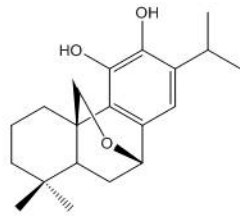


Christensen et al. (2009) *Phytother. Res.* 23, 1316-1325

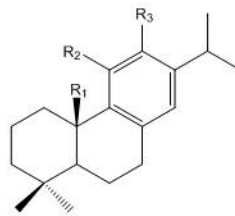
Salvia officinalis



Carnosol (1)



20-deoxo carnosol (2)

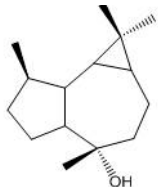


Carnosic acid (3), R₁ = COOH, R₂ = R₃ = OH

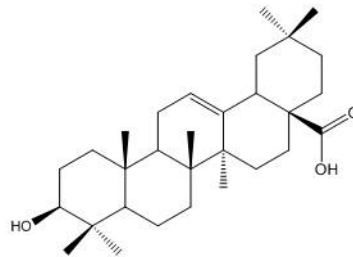
20-hydroxyferruginol (4), R₁ = CH₂OH, R₂ = H, R₃ = OH

12-O-methyl carnosic acid (5), R₁ = COOH, R₂ = OH, R₃ = OCH₃

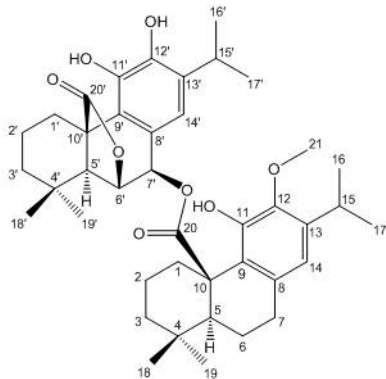
11,12,20-trihydroxy-abieta-8,11,13-triene (6), R₁ = CH₂OH, R₂ = R₃ = OH



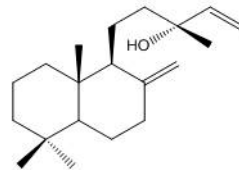
Viridiflorol (7)



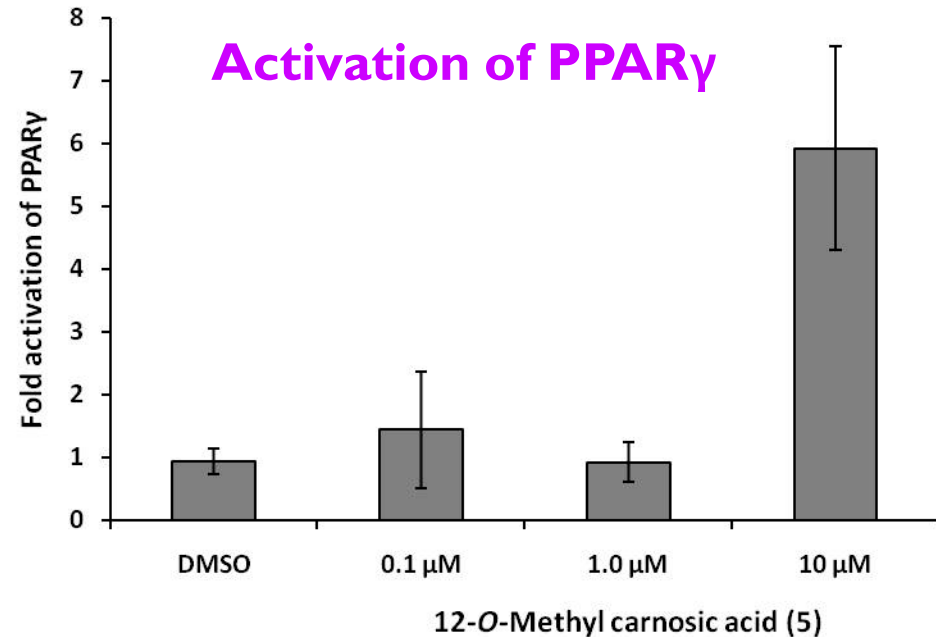
Oleanolic acid (9)



Epirosmanol ester of 12-O-methyl carnosic acid (10)



Manool (11)

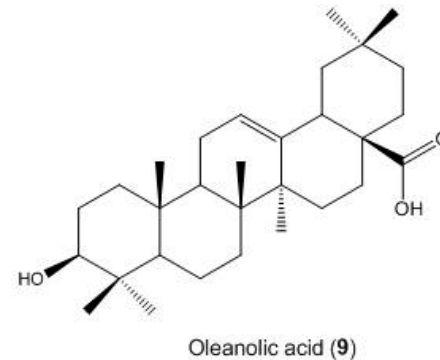
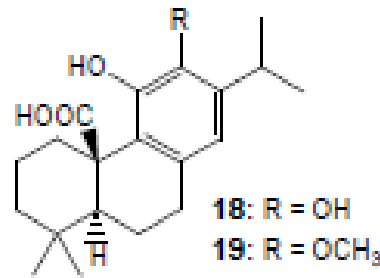
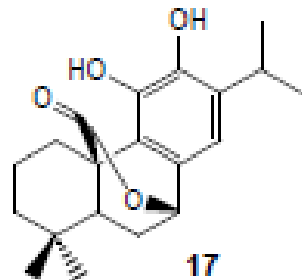


Christensen *et al.* (2010) *J. Ethnopharmacol.* 132, 127-133

Salvia officinalis

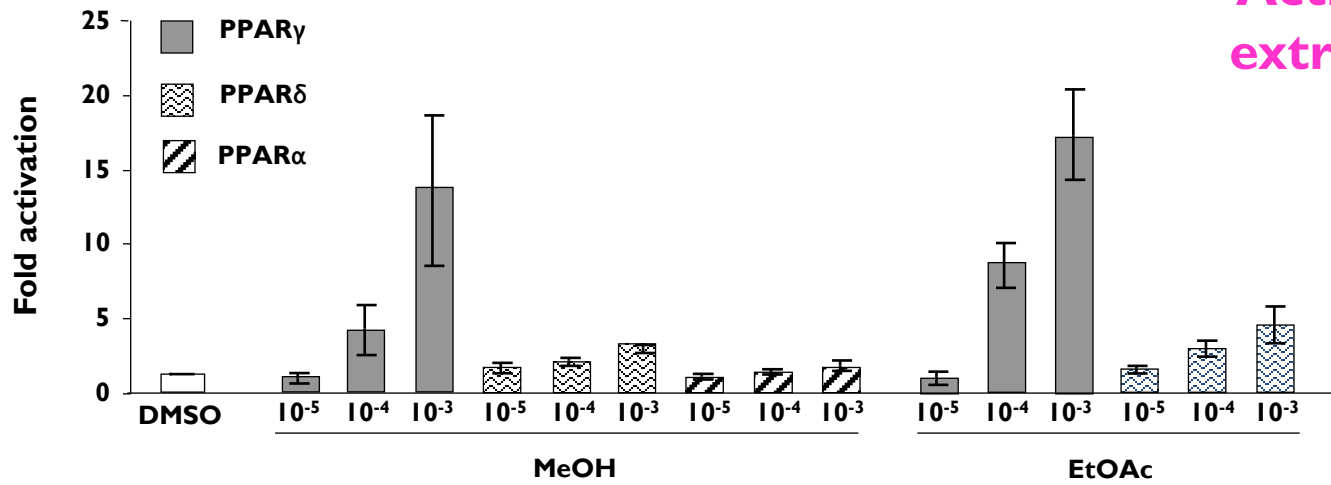
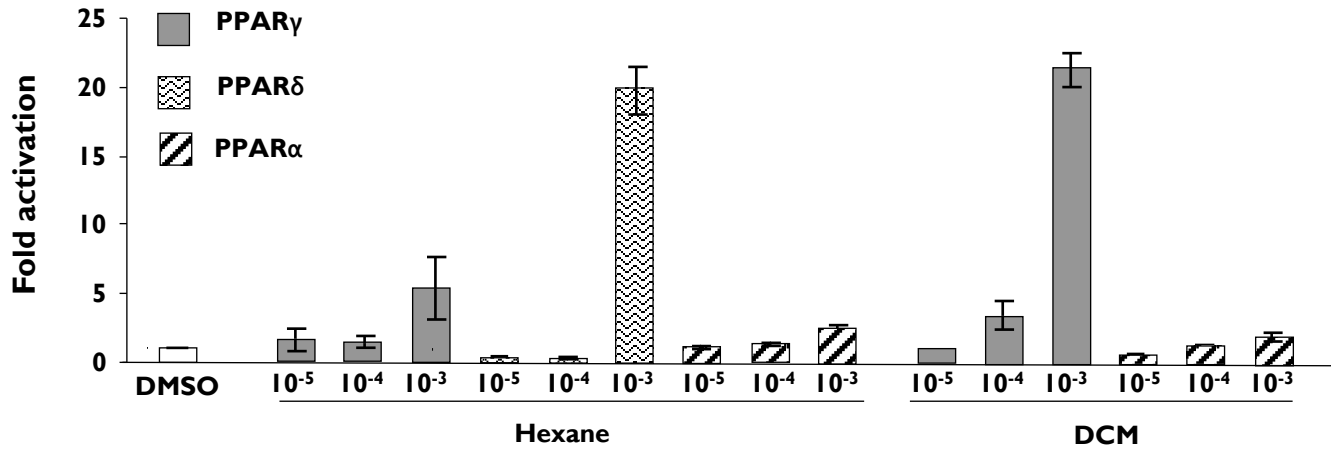


- Activation of PPAR γ with no stimulation of adipocyte differentiation
- Positive effect on glucose uptake in adipocytes



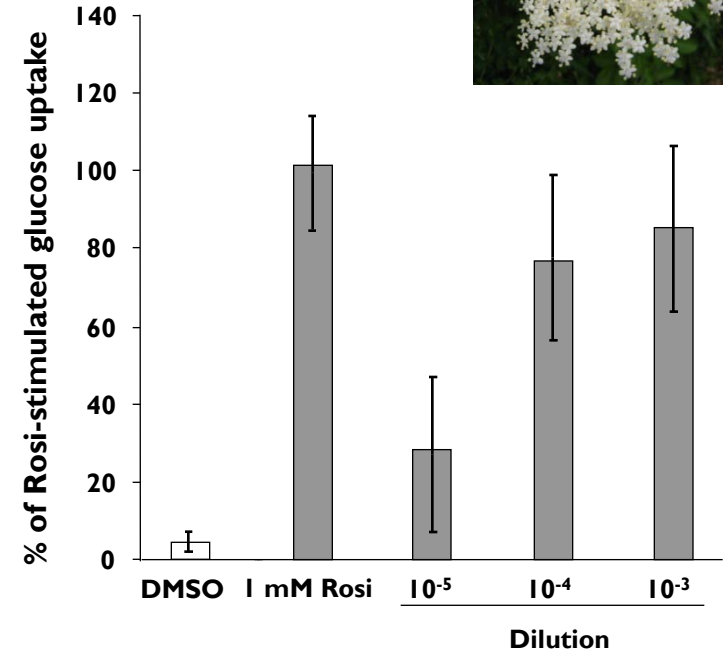
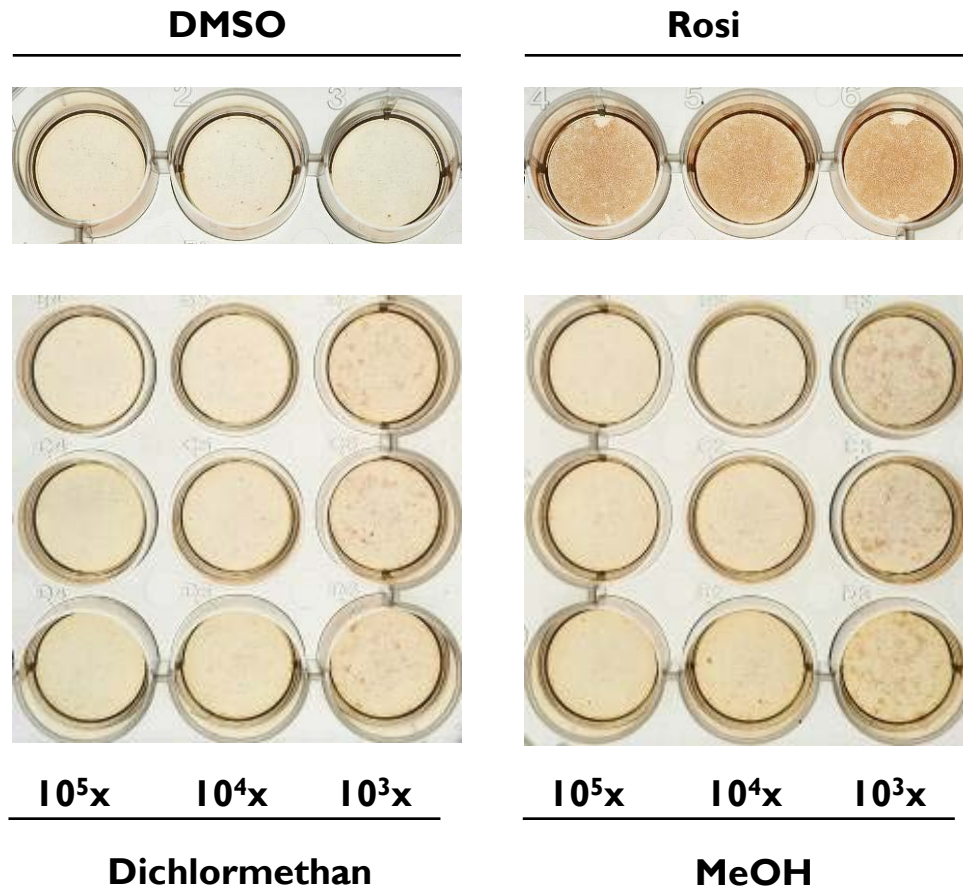
- Previously reported PPAR γ agonists from sage were found to be very weak activators whereas the PPAR α agonist oleanolic acid was found to be a dual activator of γ and α

Sambucus nigra



Activation of PPARs by extracts of elderflowers

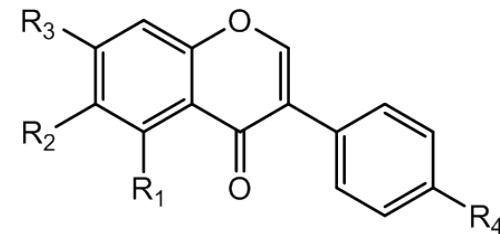
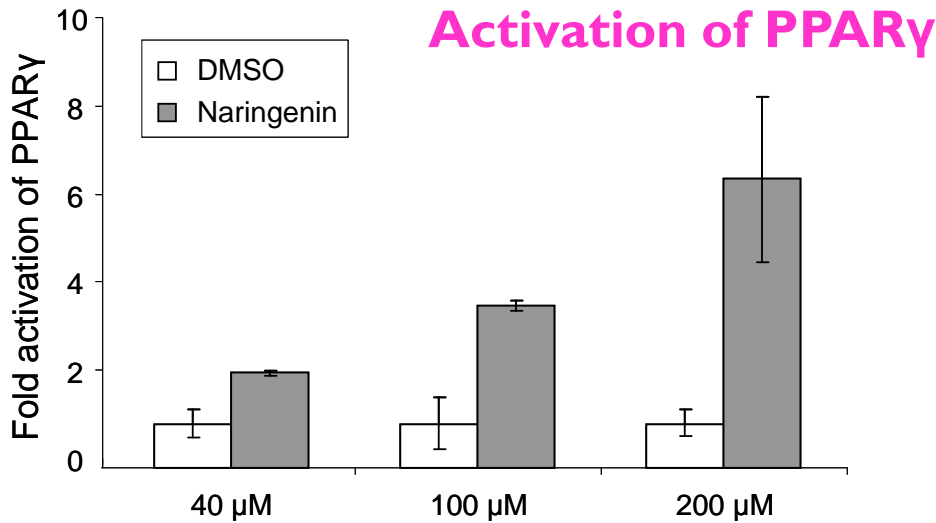
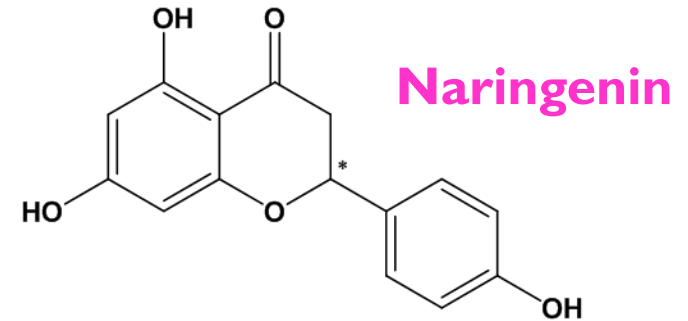
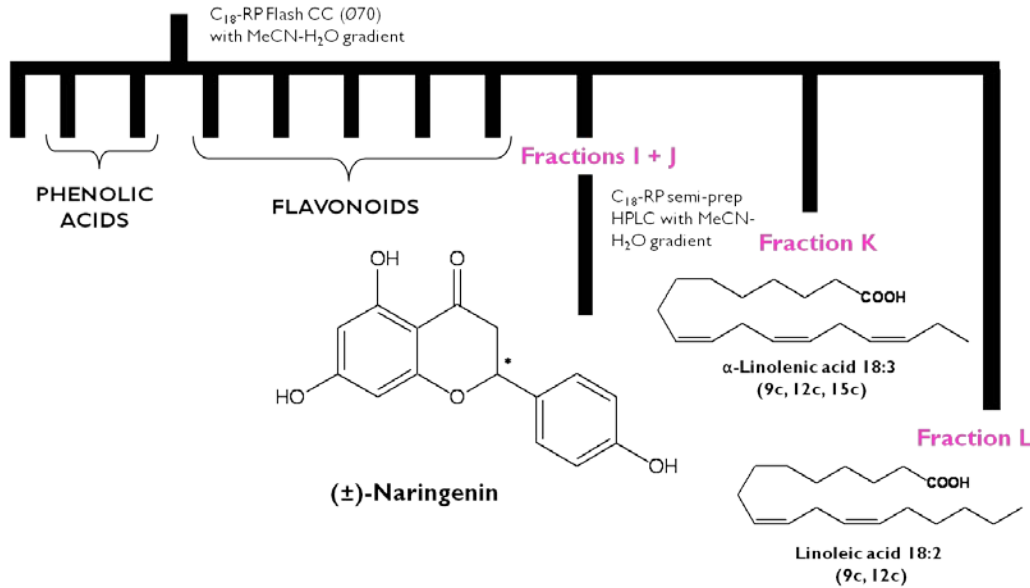
Sambucus nigra



Beneficial effect on insulin-stimulated glucose uptake by a DCM extract of elderflowers

Slight stimulation of adipocyte differentiation by extracts of elderflowers

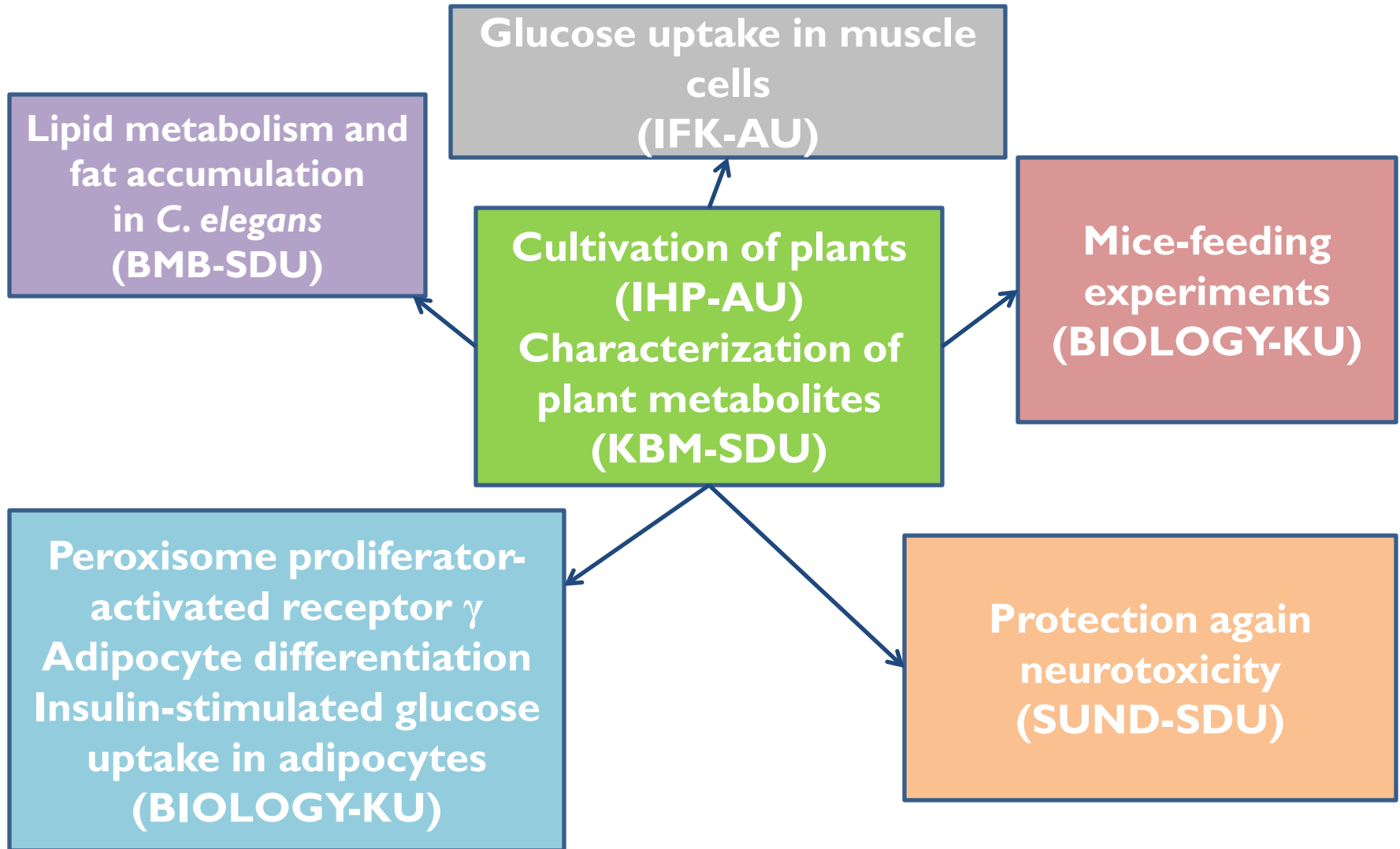
Methanolic extract of elderflowers



Genistein: R₁ = OH, R₂ = H, R₃ = OH, R₄ = OH
Daidzein: R₁ = R₂ = H, R₃ = OH, R₄ = OH
Biochanin A: R₁ = OH, R₂ = H, R₃ = OH, R₄ = OCH₃

Christensen et al. (2010) *Phytother. Res.* 24, S129-132

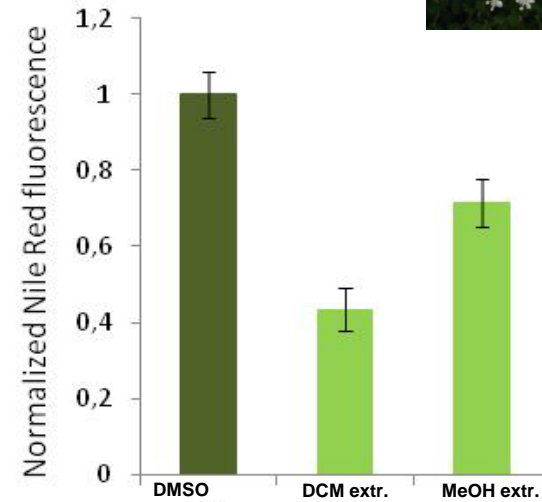
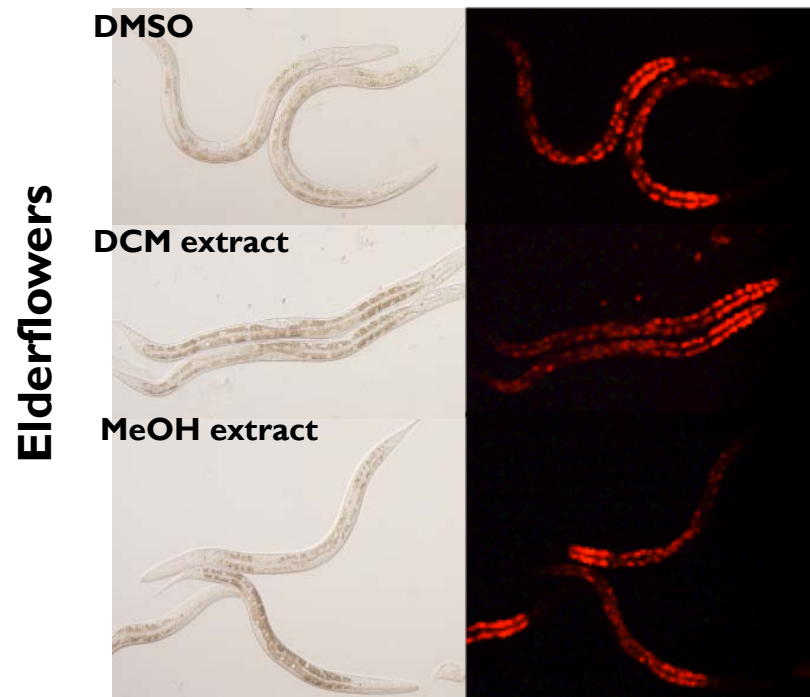
Novel screening platform



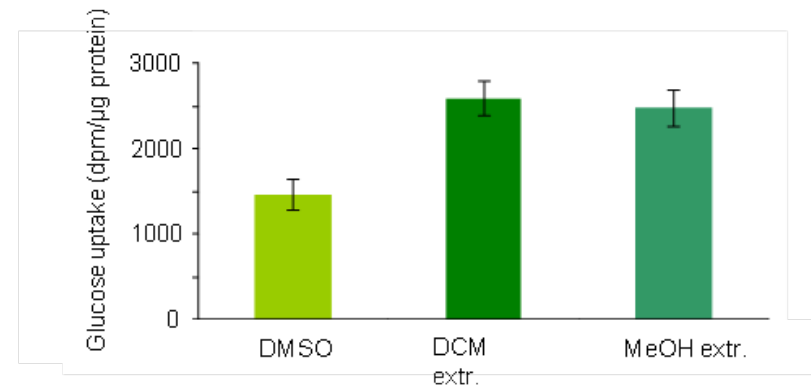
Effect on lipid metabolism and GU



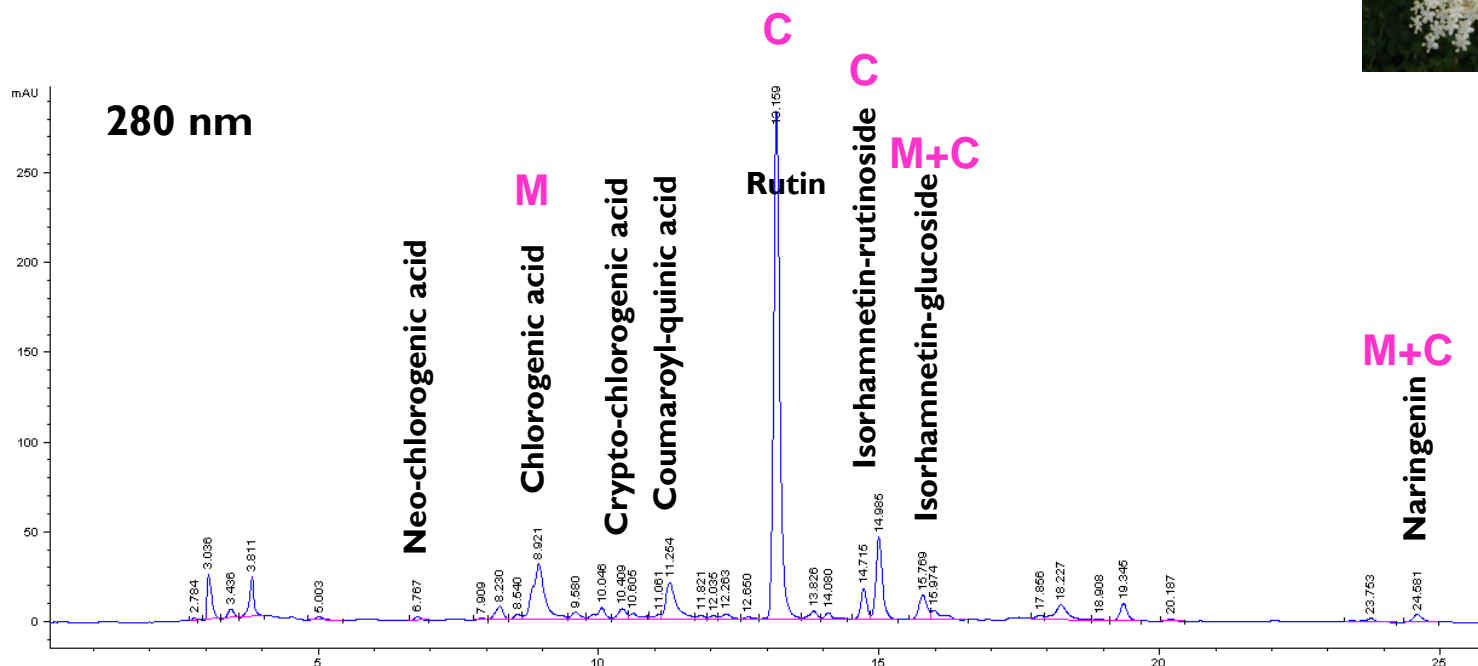
- Fat accumulation in *C. elegans*



- Glucose uptake in muscle cells



Bioactive metabolites



Elderflower MeOH extract

M = muscle cells: phenolic acids + selected flavonoids

C = *C. elegans*: flavonoids

Sambucus nigra

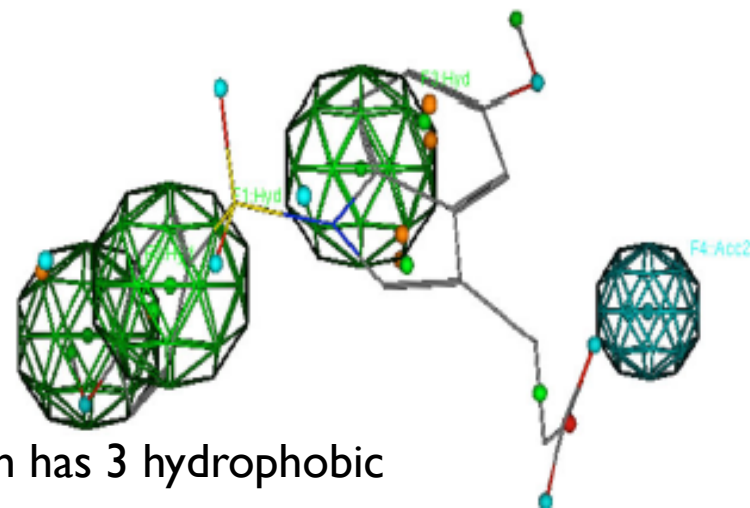


- Activation of PPAR with no or little stimulation of adipocyte differentiation
- Increase of glucose uptake in both adipocytes and porcine myotubes
- Reduction of fat accumulation in *C. elegans*
- Activation of PPAR γ is primarily caused by aglycones

In silico screening



- Virtual screening of Chinese Natural Product Database (57346 compounds) using a partial agonist pharmacophore model
 - Model was made from a compound set of 13 PPAR γ partial and selective agonists
- Hits were only recorded if they fit all the features of the pharmacophore
- **Total no. of hits = 939**



Indeglitazar is superimposed on the model here, which has 3 hydrophobic spheres and one H-bond acceptor

Petersen et al. 2011 J. Comput. Aided Mol. Des. 25, 107-116

In silico screening



- Challenge of screening:
 - Availability of compounds
 - Synthesis, isolation?
- Hit no. 24 = methyl oleanonate
 - The acid of this hit is present in the oleoresin from *Pistacia lentiscus* var. Chia
 - Ethnopharmacology hint: Chios mastic gum dispersed in water is used as an antidiabetic locally

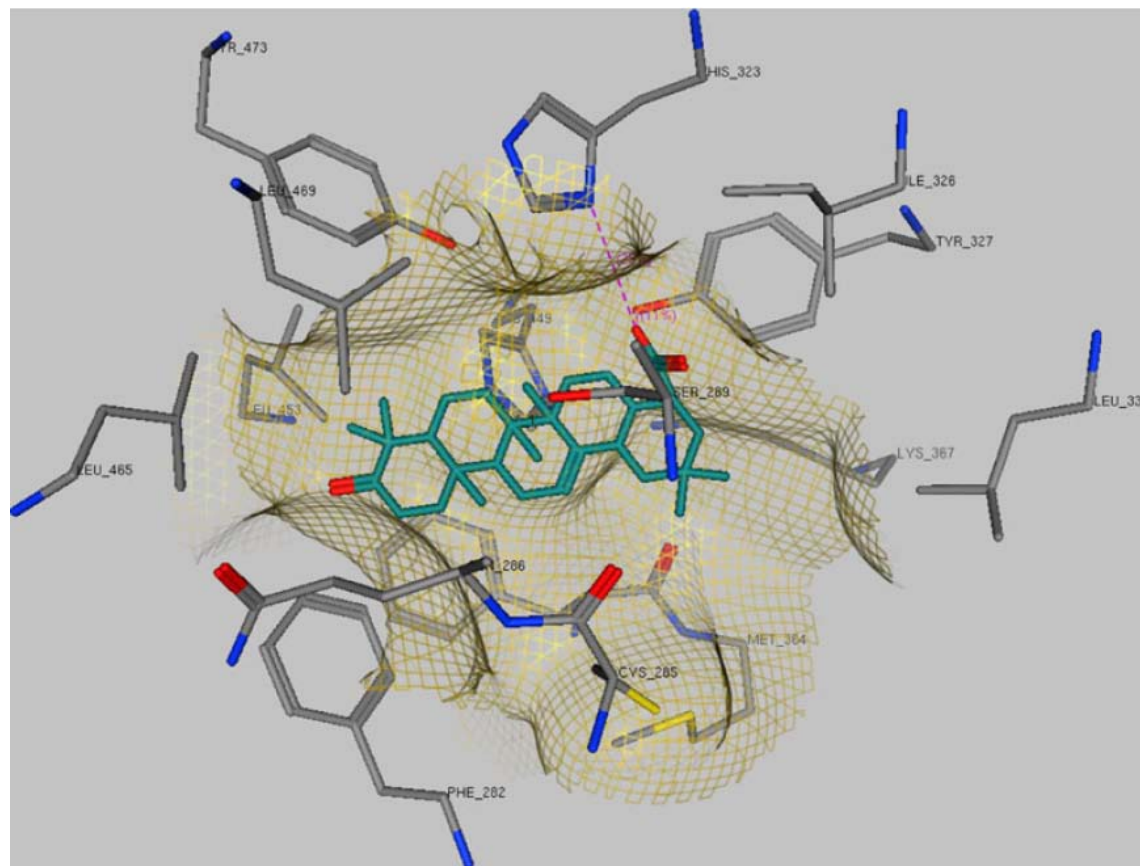


Petersen et al. 2011 J. Comput. Aided Mol. Des. 25, 107-116

Docking of oleanonic acid in the LBD of PPAR γ :

Carboxylic moiety forms H-bonds to His323 and Tyr327

The remainder of the ligand is stabilized with multiple interactions in the hydrophobic pocket

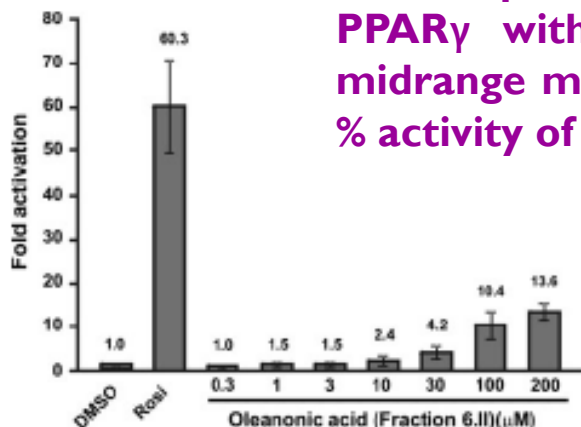


Pistacia lentiscus var. *Chia*

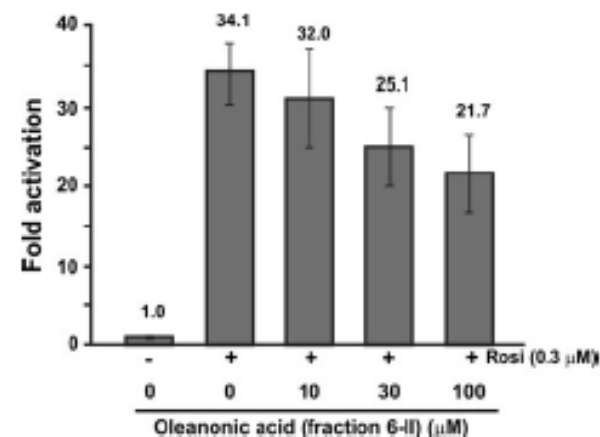


- In parallel to the *in silico* screening a bioactivity guided chromatographic isolation was performed on resins and fractions of *Pistacia* sp.
- Acidic and neutral fractions were bioactive, and further fractionations lead to the isolation of oleanonic acid

Dose-dependent activation of PPAR γ with low potency at midrange mM values giving 20 % activity of a full agonist



Competition assay with Rosi showing that oleanonic acid dose-dependently antagonizes this hence suggesting partial agonist activity



Conclusions

- For identification of PPAR agonists several approaches can be used and a combination of these is valuable
 - *Echinacea purpurea* selected on structure-activity basis
 - *Salvia officinalis* selected from reported bioactivities
 - *Sambucus nigra* selected from “chance”
 - Chios mastic gum selected from in silico screening

P = promising

P = problematic

A = adverse

R = reliable?

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Thank you for your attention!