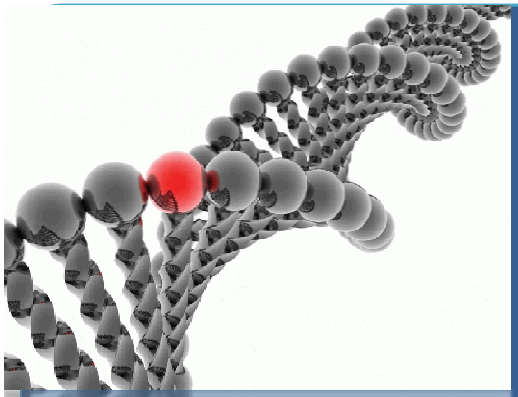
Flavones: An important scaffold for anticancer activity



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People worldwide have been using herbal medicine for the treatment, control and management of a variety of ailments since prehistoric times. There is ample archeological evidence to support the fact that primitive man used plant and herbs for medicinal purposes:

-the Sumerian clay tablet
-The Ebers papyrus
-The old testament of the Bible
-Ancient China ("The Pun-tsao", a Chinese pharmacopoeia published around 1600 BC)
-*De Materia Medica, ...*

Prior to the nineteenth century, plant medicines were administered mostly in their crude forms as infusions (herbal teas), tinctures (alcoholic extracts), decoctions (boiled extract of roots or bark), syrups (extracts of herbs made with syrup or honey) or applied externally as ointments (poultices, balms and essential oils) and herbal washes.

During the late nineteenth and early twentieth centuries, scientists began isolating, purifying and identifying active ingredients (principles) from medicinal plant extracts. This endeavor led to the discovery of some of the most important drugs that are still widely used in modern medicine:

-morphine

(isolated from *Papaver somniferum*)

-quinine

(isolated from *Cinchona* plant species)

-serpentine

(isolated from the root of the Indian plant *Rauwolfia serpentia*)

-taxol

(isolated from *Taxus brevifolius*)

-vincristine

(isolated from *Catharanthus rosesus*)

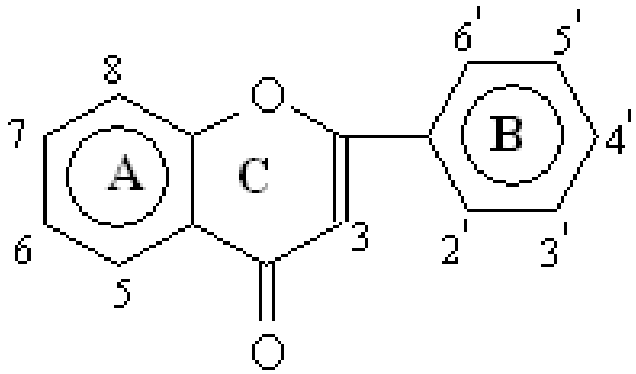
****The biologically active plant-derived natural products have served as "lead compounds" for the design, synthesis and development of novel drug compounds.**

****Medicinal plants continue to contribute significantly to modern prescription drugs by providing lead compounds upon which the synthesis of new drugs can be made.**

*****60% of the anticancer drugs and 75% of the anti-infectious disease drugs approved from 1981-2002, could be traced to natural origins.**

Flavonoids

Flavonoids are well known naturally occurring heterocyclic compounds with oxygen as a heteroatom.



Flavone ring

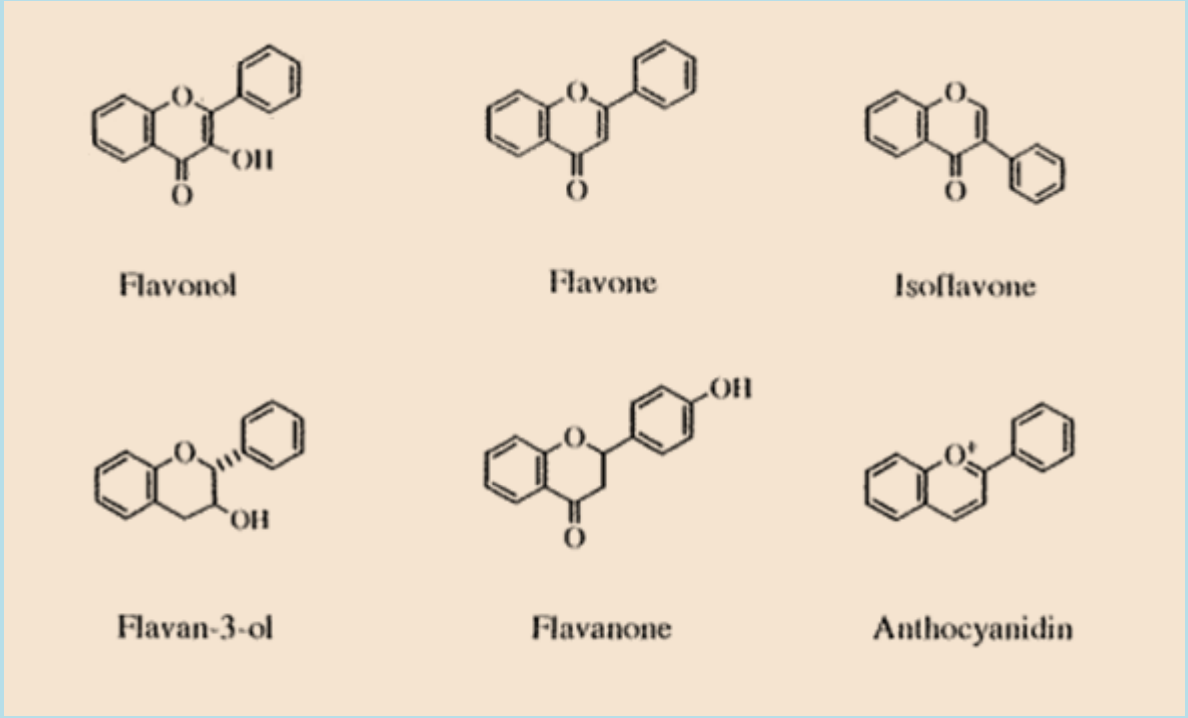
Flavonoids are polyphenols, ubiquitously found in a wide variety of edible plants, fruits, nuts, seeds and plant-derived beverages, such as juice and tea. Flavonoids are known as vitamin P or vitamin C2. However, by the 1950's the vitamin claim had been abandoned due to a lack of substantive evidence.


Flavonoids are considered an important part of the human diet, with average consumption estimates at 1.0 g flavonoids/day, and have been described as health-promoting, disease-preventing dietary supplements.

***Flavonoids represent a highly diverse class of secondary plant metabolites with about 9000 structures which have been identified up to now.**

****These compounds are found in all vascular plants as well as in some mosses.**

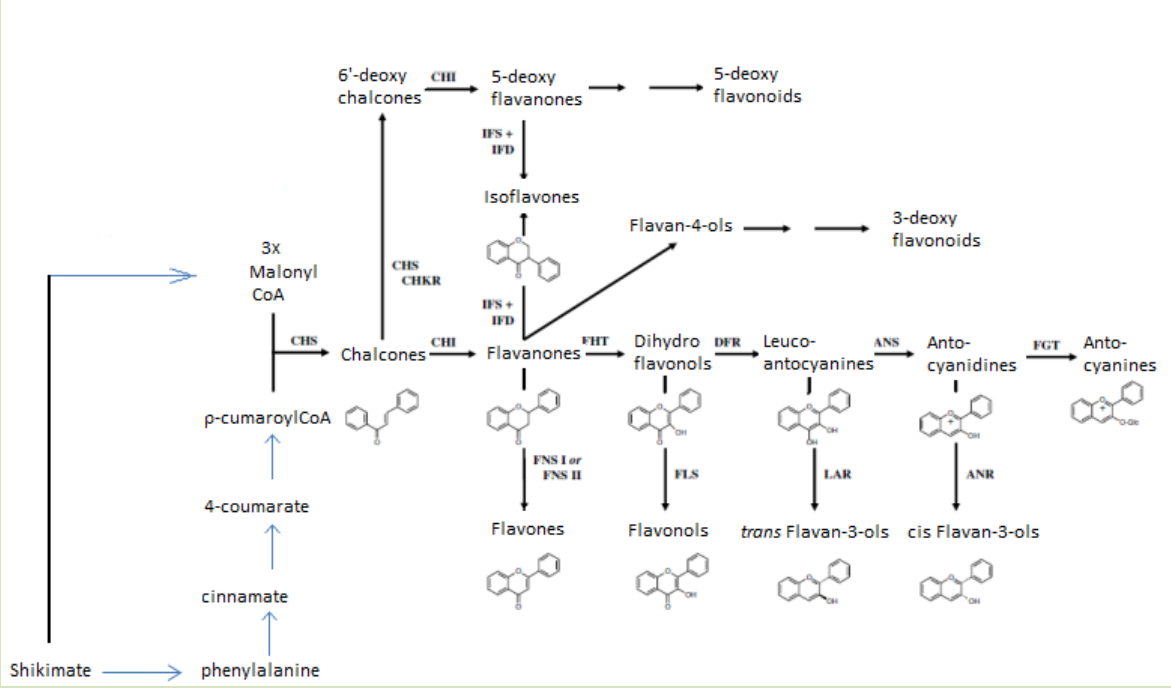
Structures of the six main classes of flavonoids:



Flavones are synthesized in plant tissues from a branch of the ***phenylpropanoid pathway***.

The key flavonoid precursors are phenylalanine, obtained via the shikimate and arogenate pathways, and malonyl-CoA, derived from citrate produced by the tricarboxylic acid (TCA) cycle.

Flavonoid end products are transported to various subcellular or extracellular locations, with those flavonoids involved in pigmentation generally being transported into the vacuole.

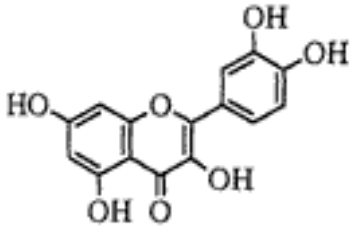


Biosynthesis of Flavones: CHS, chalcone synthase; CHKR, chalcone reductase; CHI, chalcone isomerase; FHT, flavanone 3-β-hydroxylase; DFR, dihydroflavonol 4-reductase; ANS, antocyanidin synthase; FGT, flavonoid glicosyltransferase; FNS, flavon synthase; FLS, flavonol synthase; LAR, leucoantocyanidine reductase; ANR, antocyanidine reductase; IFS, isoflavone synthase; IFD, isoflavone dihydratase

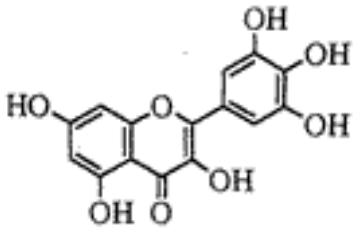
In plant tissues, flavones are found conjugated to sugars, primarily glucose, rhamnose and rutinose.

Most conjugation occurs at the 3' position of the B ring, although it can also occur frequently at the 7 and 4' positions.

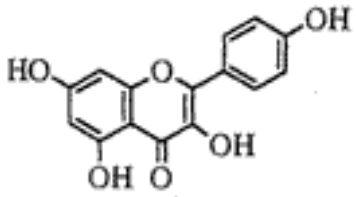
Structures of the main dietary flavonols and flavones:



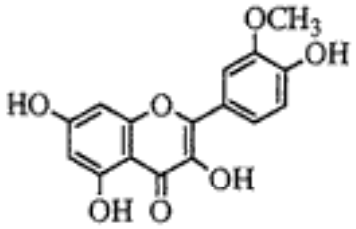
Quercetin



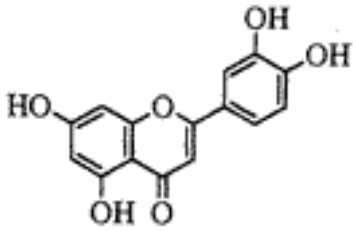
Myricetin



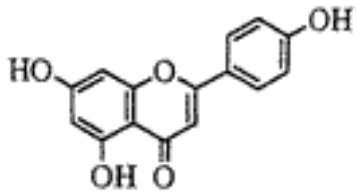
Kaempferol



Isorhamnetin



Luteolin

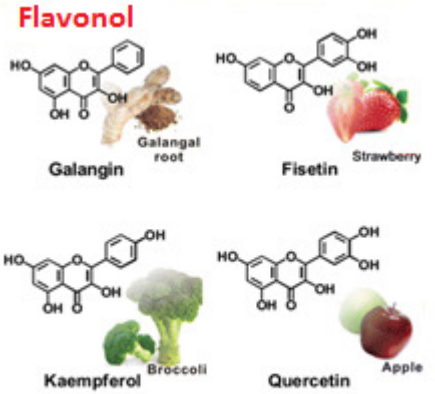


Apigenin

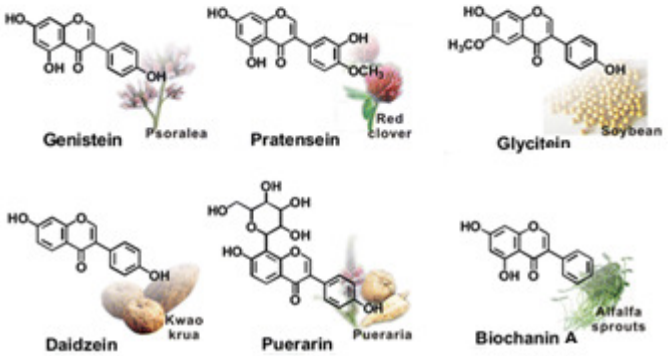
The major flavone aglycones in fruits and vegetables

Flavones

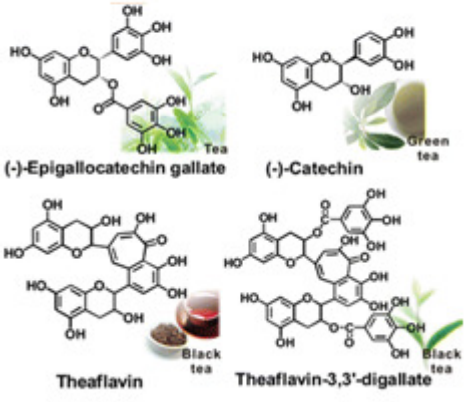
Flavonol



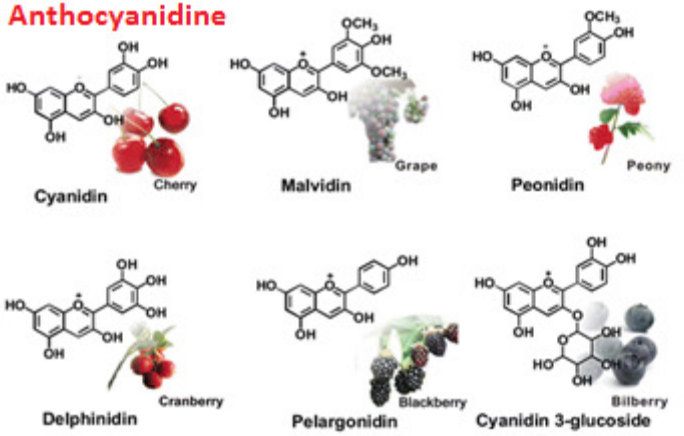
Isoflavone



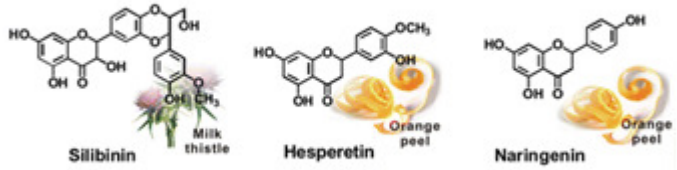
Flavan-3-ol



Anthocyanidine



3-Hydroxy-flavanone



Flavones have been shown to possess a variety of biological activities at nontoxic concentrations in organisms :

- Effect on Enzymes
- Hypoglisemic Effect
- Effect on central nervous system
- Effect on autonomic nervous system
- Cardiovascular system effects
- Antiallergic effects
- Effect on Gastrointestinal system
- Antineoplastic
- Antiviral
- Antiinflammatory
- Antifungal
- Antithrombocyter
- Antibacterial
- Dopaminergic
- Antiproliferative effects, ...

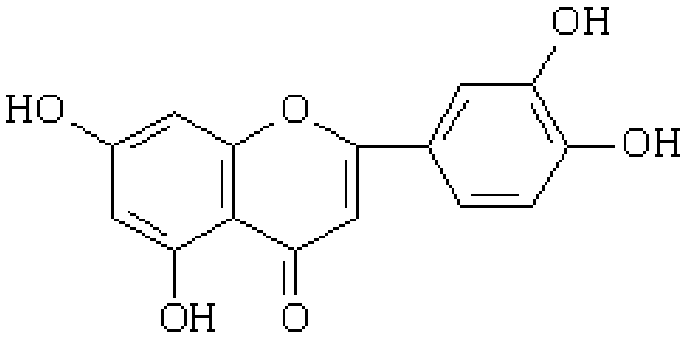
The role of dietary flavonoids in cancer prevention is widely discussed. Compelling data from laboratory studies, epidemiological investigations, and human clinical trials indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy. Many mechanisms of action have been identified:

- carcinogen inactivation,
- antiproliferation,
- cell cycle arrest,
- induction of apoptosis and differentiation,
- inhibition of angiogenesis,
- antioxidation
- reversal of multidrug resistance or a combination of these mechanisms.

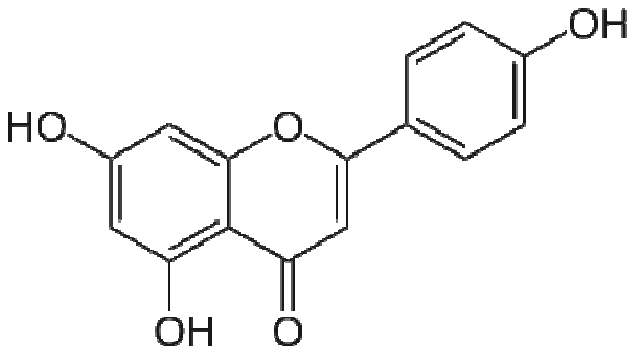
The health benefits of flavonoids are usually linked to two properties:

(i) inhibition of certain enzymes; **(ii) antioxidant activity**

apigenin, luteolin is found to inhibit class I histone deacetylases (HDACs) leading to cell cycle arrest in prostate cancer cell lines and inhibit tumor growth of mice xenografts.



Luteolin

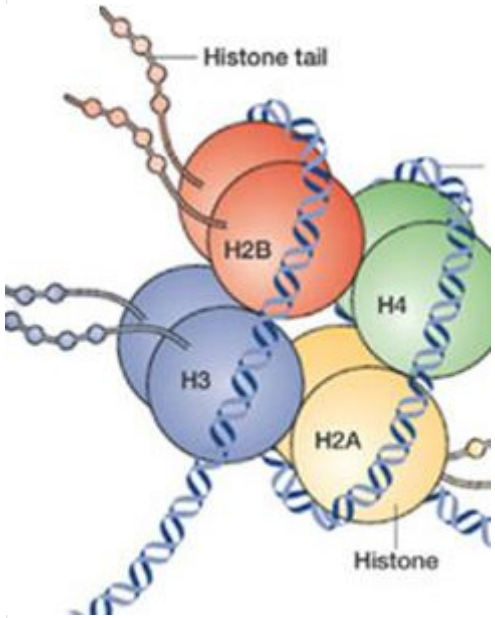


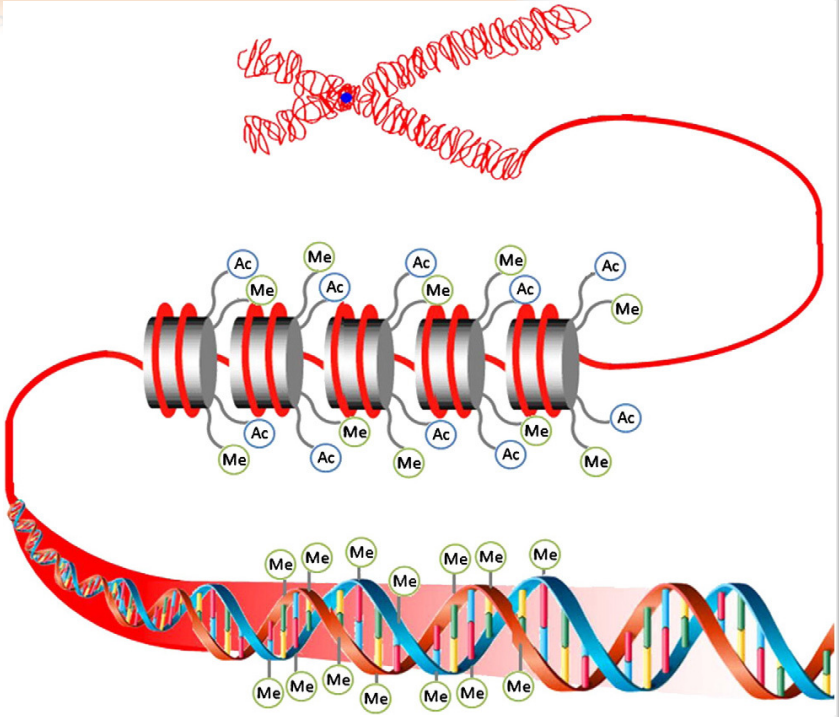
Apigenin

Many HDACs were involved in human cancers because overexpression of particular HDACs was found in some cancers, for example, HDAC6 in breast cancer , HDAC2 in colorectal cancer , HDAC1 in prostate cancer, ...

Histone Proteins

In eukaryotic cells, DNA is packaged with histones and non- histone proteins. This structure is called as chromatin. The basic chromatin proteins are histones. There are five types of histone proteins including H1, H2A, H2B , H3 and H4. Histone proteins are composed of nucleosome structure .





Packaging of DNA into chromatin and the epigenome.
Abbreviations: Ac, acetyl; Me, methyl.

DNA is tightly coiled around cores of eight histone proteins to form nucleosomes. Post translational modifications of these histone proteins (acetylation and methylation) as well as direct modification of DNA help control the compression of this structure and enable transctiptional factor access to DNA.

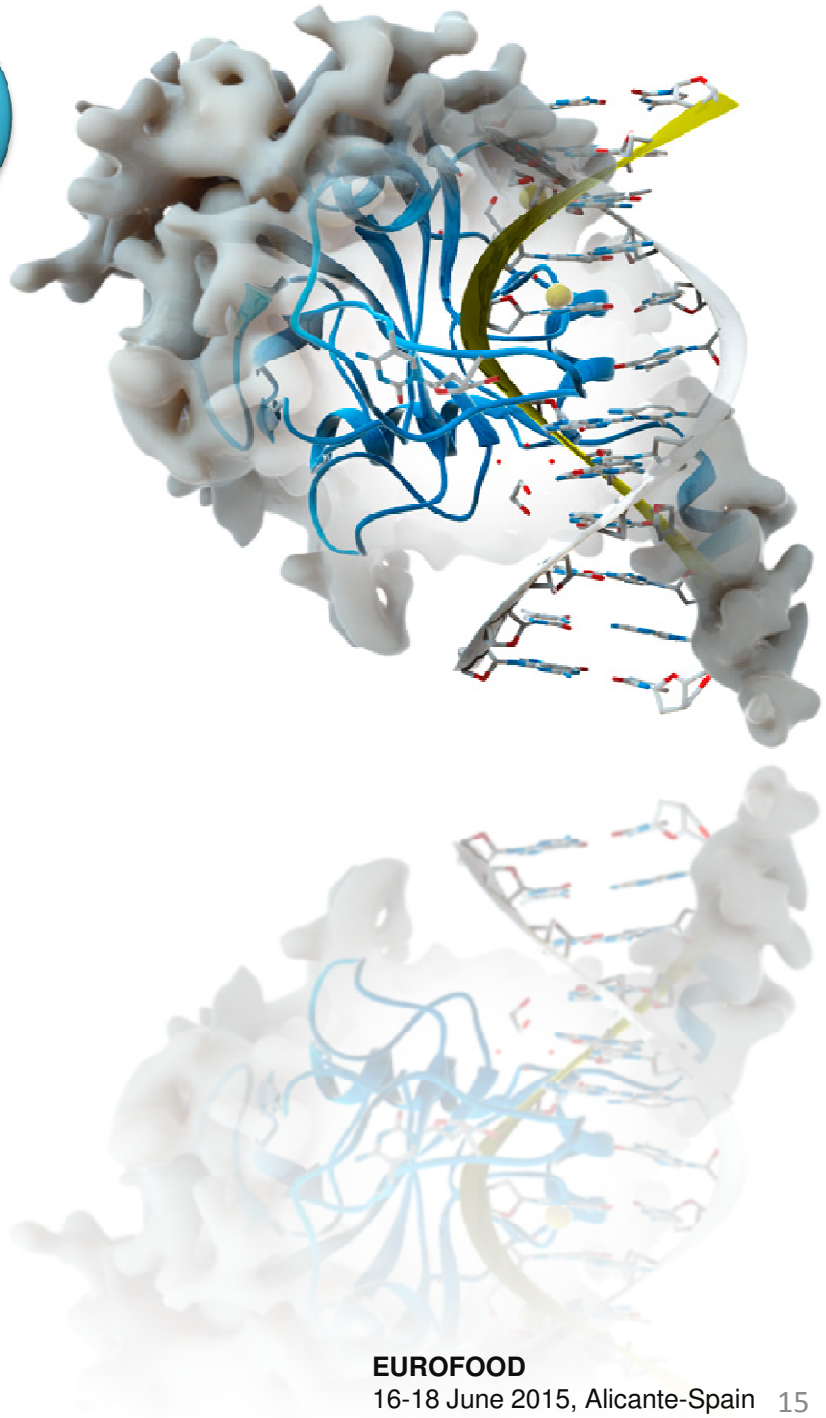
***** Silencing of tumor suppressor genes by DNA methylation and histone deacetylation are important in the pathogenesis of cancer.**

HDACs (histone deacetylases) are a family of enzymes involved in the regulation of a number of cellular processes :

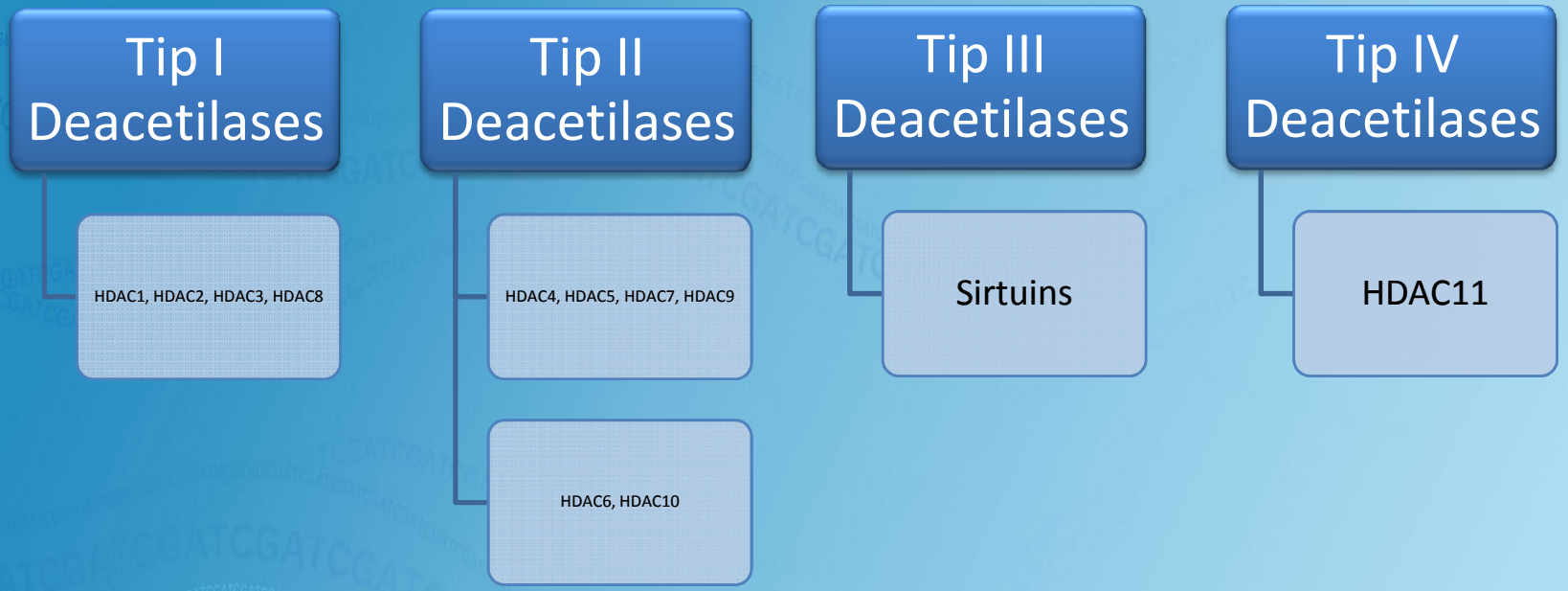
- cell proliferation
- apoptosis
- inhibition of angiyogenezis
- assembly of the cytoskeleton
- the control of gene expression through regulation of transcription

Histone acetylation activates only the transcription of certain genes (~ 2% of the expressed genes).

Expressed genes inhibit tumor growth.



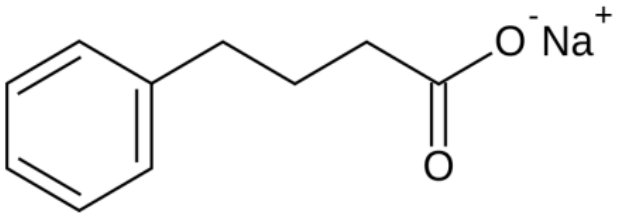
HDACs:


Tip I Deacetilases
HDAC1, HDAC2, HDAC3, HDAC8
Tip II Deacetilases
HDAC4, HDAC5, HDAC7, HDAC9
HDAC6, HDAC10
Tip III Deacetilases
Sirtuins
Tip IV Deacetilases
HDAC11

HDACIs on cancer cells

Acetylation and deacetylation of histones are significant importance in tumor genesis and progression. Inhibition of HDACs has become a promising direction for cancer therapy.

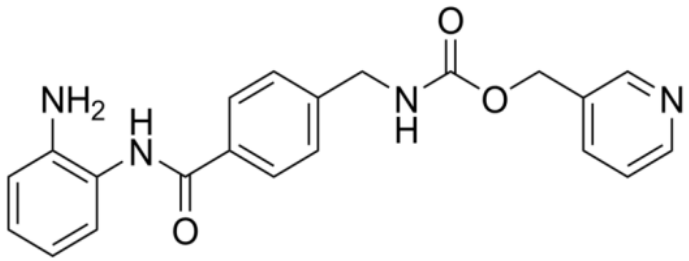
**HDACIs have been used as a new class of anticancer agents in clinical trials, and have been studied extensively in the laboratory. Clinical studies have shown that histone hyperacetylation can be achieved safely in human, and that treatment with such agents is plausible.



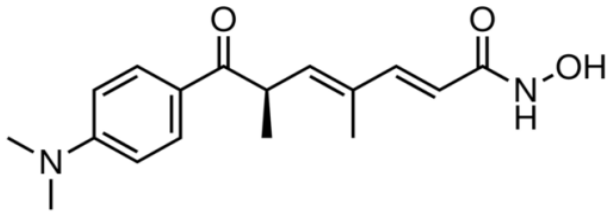
Natrium phenyl butirat

HDACIs include a range of naturally occurring and synthetic compounds that differ in terms of structure, function, and specificity.

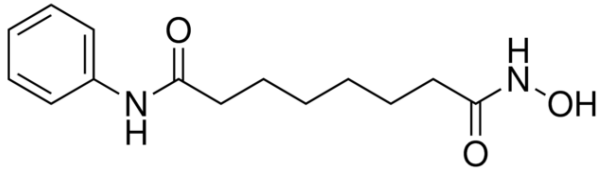
- Short chain oil acids
- Hydroxamic acids
- Benzamides
- Cyclic tetrapeptides
- Electrophilic ketons



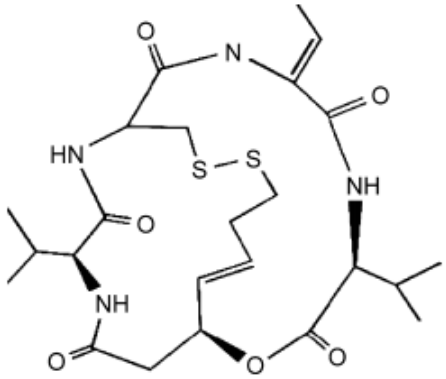
Entinostate (MS-275)



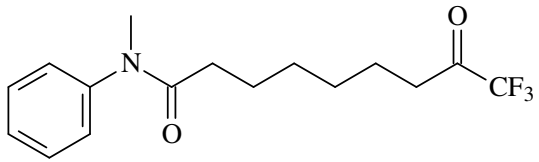
Trichostatin A (TSA)



Suberoylanilide hydroxamic acid (SAHA)



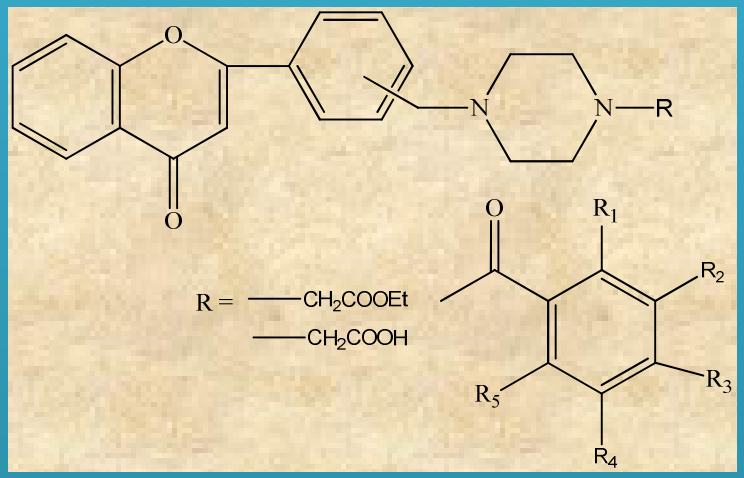
Depsipeptit (FK-228)



Trifluoromethyl keton

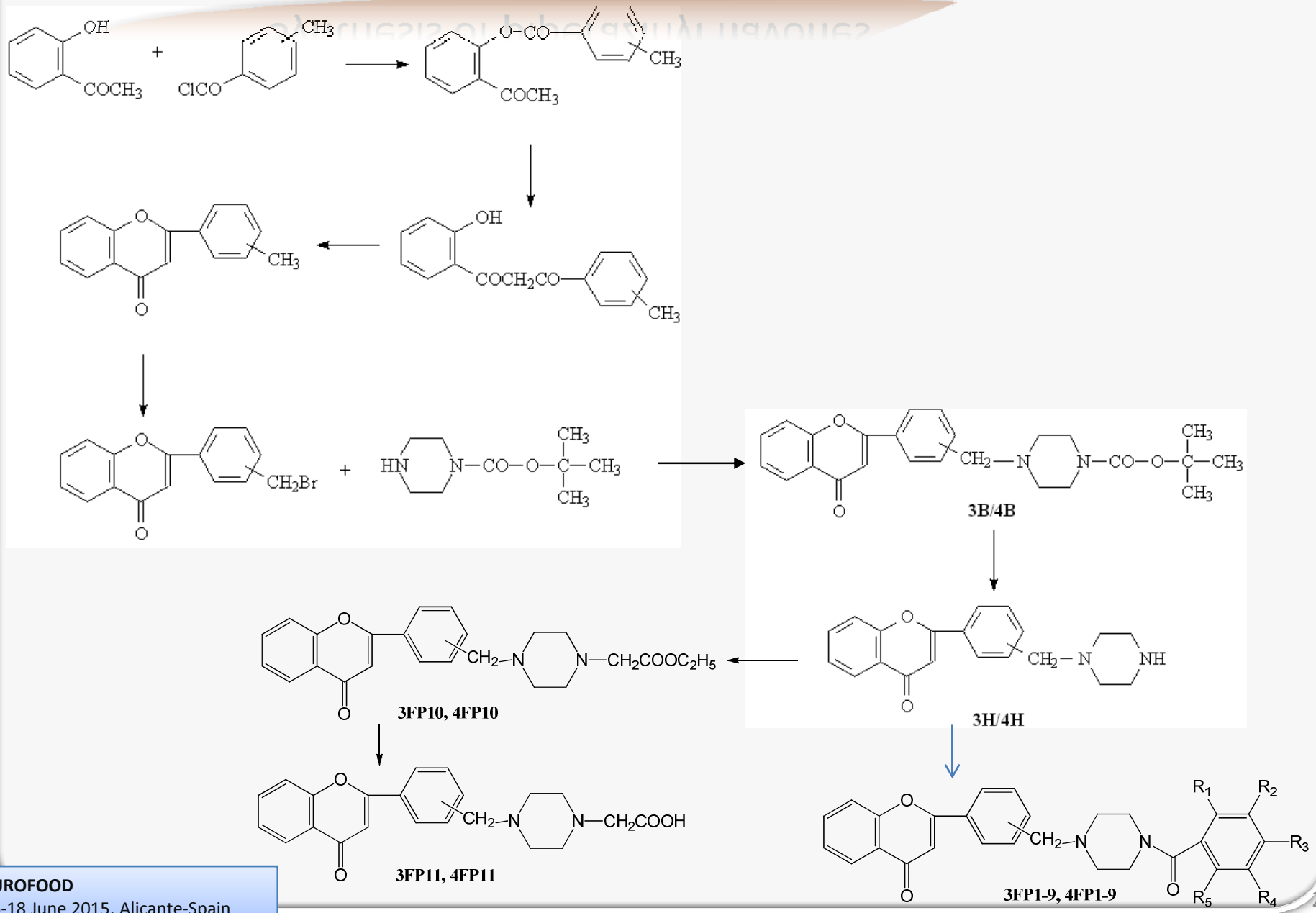
Naturally obtained flavone moiety having a variety of biological activities can be taken as lead compound for the synthesis of synthetic flavone derivatives with different functional groups at different positions of flavone skeleton.

In this study, we have synthesized **piperazinylflavone derivatives** and elucidated possible structure–activity relationships of them that might lead to new anticancer drug discovery. The first part of our work focuses on synthesizing a new series of benzamido / acetic acid / acetic acid ethyl ester substituted piperazine containing flavone derivatives [3FP1-11 and 4FP1-11] with the purpose of increasing the antitumor activity of flavones by the aid of piperazine molecules.

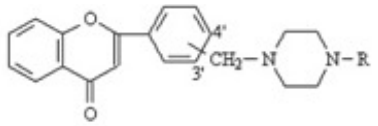


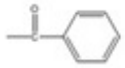
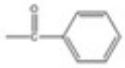
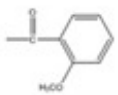
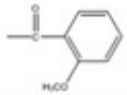
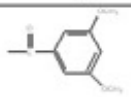
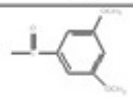
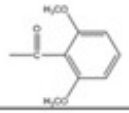
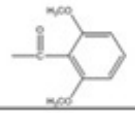
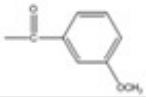
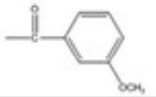
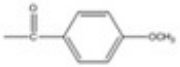
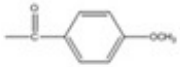
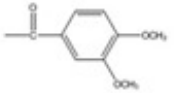
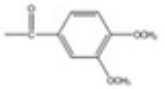
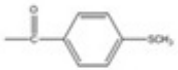
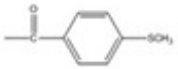
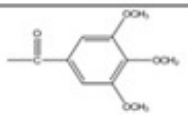
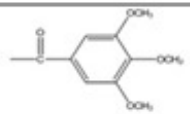
Piperazinyl flavones
(3FP1-11 and **4FP1-11)**

Synthesis of piperazinyl flavones



Piperazinyl flavones (3FP1-11; 4FP1-11)



Code	Position	R	Code	Position	R
3FP1	3'		4FP1	4'	
3FP2	3'		4FP2	4'	
3FP3	3'		4FP3	4'	
3FP4	3'		4FP4	4'	
3FP5	3'		4FP5	4'	
3FP6	3'		4FP6	4'	
3FP7	3'		4FP7	4'	
3FP8	3'		4FP8	4'	
3FP9	3'		4FP9	4'	
3FP10	3'	$CH_2COOC_2H_5$	4FP10	4'	$CH_2COOC_2H_5$
3FP11	3'	CH_2COOH	4FP11	4'	CH_2COOH

Results and discussion

Initial screening of all compounds were performed by determining potential anti-growth effects of 22 different piperazinyl flavone (**FP)** compounds in two hepatocellular carcinoma cell lines, Huh7 and PLC respectively. For initial screening with Sulforhodamine B assay (SRB), compounds were used at a concentration of 10 µM (Figure 1a, 1b).

3'-substituted compounds (**3FP**) were far more cytotoxic than 4'-substituted compounds (**4FP**) against both Huh7 and PLC cell lines.

Huh7 cell line was more sensitive to all tested compounds compared to PLC cell line.

Figure 1.a

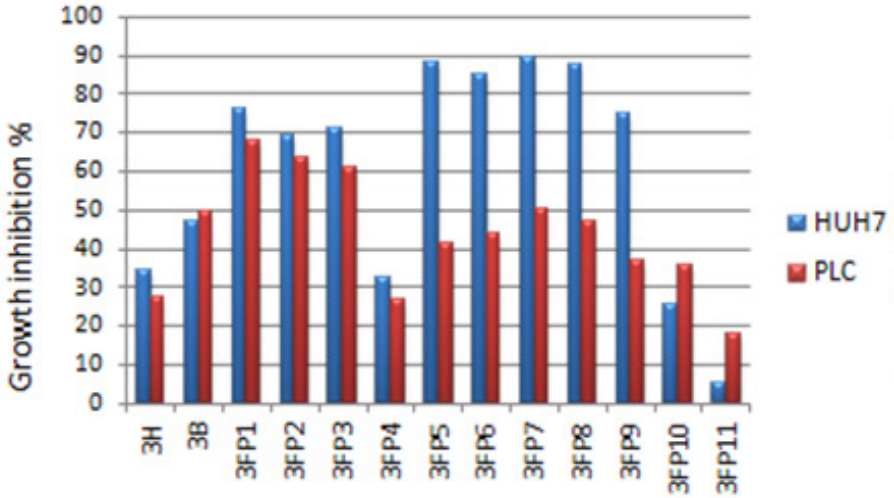
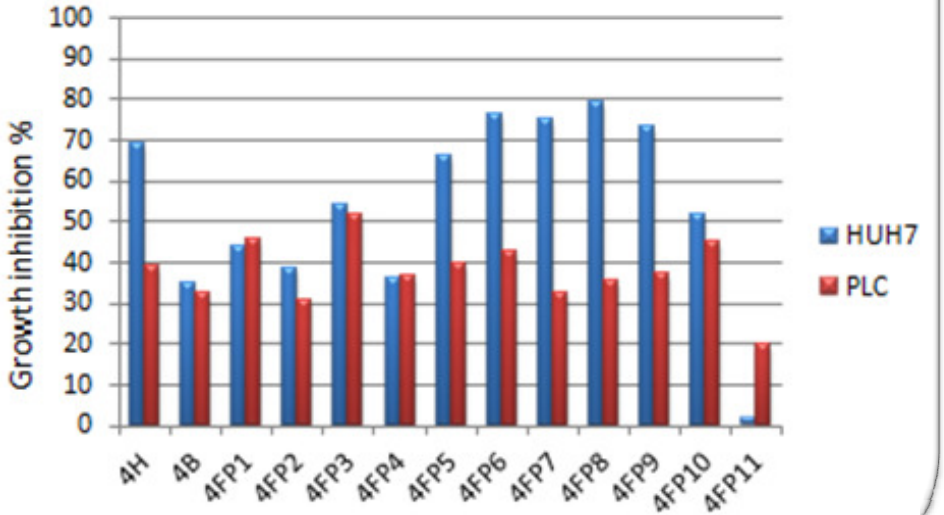


Figure 1.b



Results and discussion

We calculated IC_{50} values for the most promising five compounds (**3FP1, 3FP5, 3FP6, 3FP7, 3FP8**) in 9 different cancer cell lines due to their high cytotoxicity (Table 2).

Table 2. IC50 values of the five compounds **3FP1, 3FP5, 3FP6, 3FP7, 3FP8** and Entinostat in 9 different cancer cell lines.

		3FP1		3FP5		3FP6		3FP7		3FP8		Entinostat	
Cell line	Cells/well	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2	IC_{50} (μM)	R^2
Mcf7	2000	7,9	1,0	8,4	1,0	5,5	0,9	3,7	1,0	4,0	0,9	-	-
Huh7	2000	7,5	1,0	8,0	1,0	5,3	1,0	3,0	1,0	3,4	0,9	1,5	1,0
HepG2	4000	6,6	0,9	5,1	0,8	0,3	0,9	0,010	0,8	0,1	0,7	-	-
Hep3B	6000	11,5	1,0	8,0	1,0	6,0	1,0	4,9	1,0	4,7	0,8	-	-
Plc	6000	10,9	1,0	8,4	1,0	7,9	1,0	6,1	1,0	6,1	0,9	1,8	1,0
Snu449	8000	31,1	1,0	16,3	0,9	41,0	1,0	14,0	0,9	72,4	0,8	-	-
SK-BR3	10000	10,6	1,0	11,6	1,0	14,0	0,9	7,6	1,0	9,7	1,0	-	-
HCT 116	4000	12,8	1,0	10,9	1,0	14,1	1,0	8,4	1,0	10,1	0,9	-	-
HCT116 p53 deficient	4000	18,0	0,9	14,2	0,9	12,0	1,0	5,2	1,0	11,3	0,9	-	-

Results and discussion

Since all compounds were found to be more cytotoxic in Huh7, this cell line was chosen for further analysis to test potential HDACi (HDAC inhibitor) activity of the compounds (Figure 2).

Acetylated histone or acetylated tubulin levels were detected by Western blot technique by using a specific antibody developed against acetylated tubulin or histone protein (H3 or H4). Four compounds (**3FP1, 3FP5, 3FP6, 3FP8**) were chosen for their histone deacetylase inhibition capacity besides their cytotoxicity.

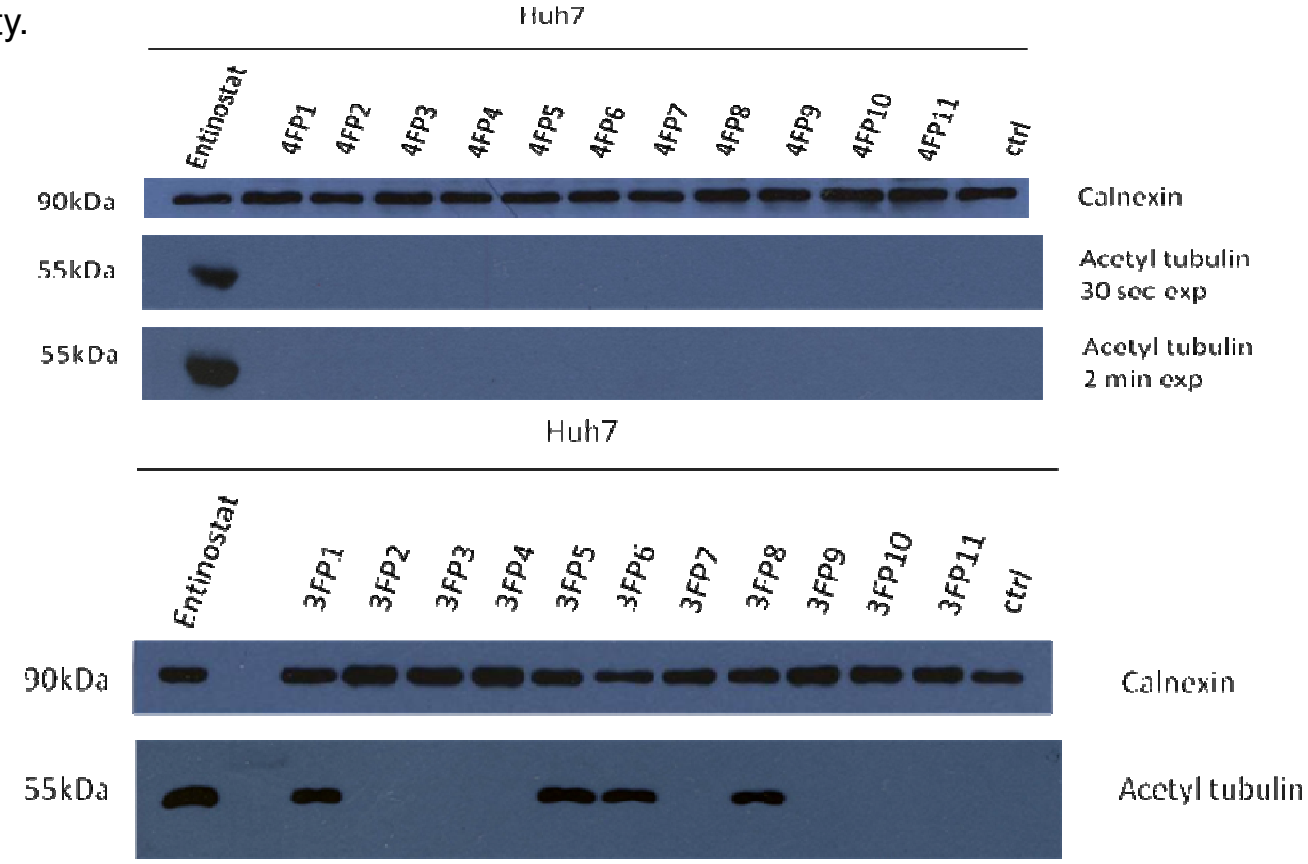


Figure 2.

Results and discussion

TSA was able to increase tubulin acetylation compared to untreated Huh7 cells; while main compounds **3H**, **4H**, **3B** and **4B** could not trigger an increase in acetylated tubulin levels meaning that the main compounds had no inhibitory effect on HDACs (Figure 3).

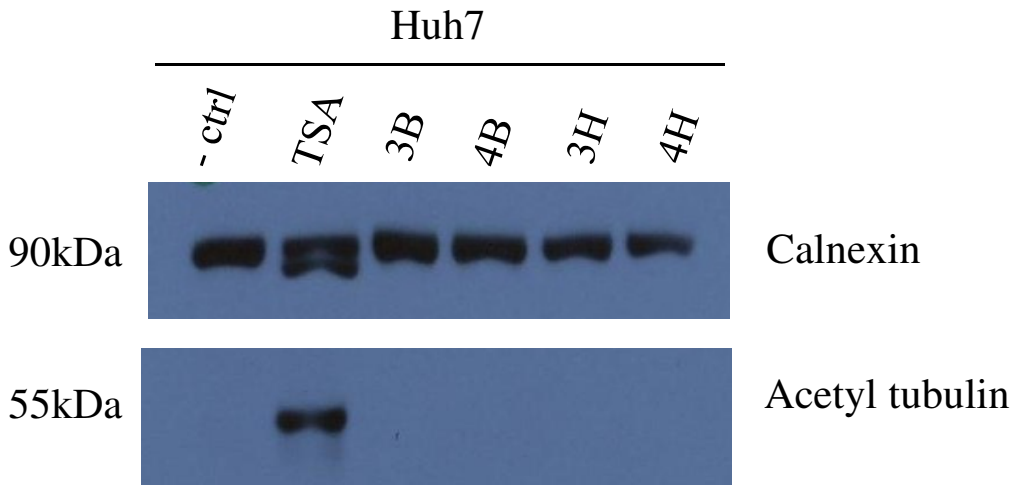


Figure 3. Tubulin acetylation levels in Huh7 cells after treatment with 10μM of main compounds or 2μM of TSA. Calnexin is used for loading control.

Conclusion

Five of the 3' -flavone derivatives (**3FP1**, **3FP5**, **3FP6**, **3FP7**, and **3FP8**) demonstrated significant cytotoxic activity with an average IC_{50} value of 0.01-14.1 µM against several cancer cell lines originated from different tissues such as liver, breast and column.

***Flavone-3' -yl pharmacophore was far more effective compared to flavone-4' -yl.

3'-Flavone derivatives would be more cytotoxic against non-aggressive tumors because HepG2 is considered as a representative of such tumors. If we put aside Snu449 IC_{50} values, **3FP7** is the most cytotoxic agent right after adriamycin which is a well known anticancer drug.

However, mechanism of action of **3FP7** was different from other strong cytotoxic derivatives because it did not show any HDACi activity on alpha tubulin deacetylases or histone 6 deacetylases.

Among twenty two FP derivatives, four compounds; **3FP1, 3FP5**, **3FP6 and 3FP8** had convincing histone deacetylase 6 inhibitory activity with acetyl alpha-tubulin accumulation.

Finally:

We suggest that 3FP compounds are potential cytoplasmic HDAC inhibitors that are selective for HDAC6 and sirtuin 2.

HDAC6 with specific structure and biological function plays a significant role in the carcinogenesis, progression and metastasis of tumors. As the study of HDAC6 is in an initiation phase, **HDAC6 will be a hot topic for cancer therapy**.

Because of their unchanged cytotoxic effect on HCT116 isogenic cell lines, 3' -flavone derivatives may induce p53 independent cell death mechanism/s. However, further studies should be performed on **3FP1**, **3FP5**, **3FP6**, **3FP8** compounds to have a more clear perspective on their chemotherapeutic activities against cancer as they are very promising HDACIs.

Thank you for your attention

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