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FLAVONOIDS IN ECOTOXICOLOGICAL CONTEXT. THE HYDROPHOBICITY ROLE IN COMMON QSARs

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ABSTRACT: The naturally occurring flavonoids are be the primary focus of this review, with occasional reference to synthetic compounds; although not exhaustive, it is intended to acquaint the reader with this interesting group of natural plant compounds, since there has been in recent years a major rekindling of interest in pharmacognosy, while flavonoids turn out to be present in many natural therapeutically utilized products. Appropriate discussion on chemical-biological interaction was as well presented within the quantitative structure-activity relationship analysis, with which occasion the Non-Gaussian-Leave-Out method of molecular screening was adopted, while emphasizing on the role of hydrophobicity, of it order dependence and of the structural electronic factors in providing acceptable Multilinear correlations.

Keywords: Toxicity, inhibitory activity, biosynthesis, QSAR.

1. INTRODUCTION

Flavonoids are natural components of human and animal diet and have been shown to exert different biological effects, such as antiviral, anti-inflammatory, antimutagenic and anticarcinogenic functions. This activity is reported to result partly from their antioxidant and antiradical properties (Sugihara, N et al., 1999). Flavonoid compounds are one of the most analyzed groups of secondary metabolites in higher plants. The main reason for the interest in flavonoids is that they are major constituents of plant pigments.

Over 4000 structurally unique flavonoids have been identified in plant sources (Halliwell B et al., 1992). Primarily recognized as the pigments responsible for the autumnal burst of hues and the many shades of yellow, orange, and red in food and flowers (Bourdillat B, 1988). The flavonoids are found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers, as well as tea and red wine.

Many natural flavonoids show anti-inflammatory properties, based on several mechanisms including the effect on free radical formation or radical scavenging (Cotelle, N., et al., 1996).

They are components of citrus fruits (Kefford JF and Chandler BV, 1970) and other food sources (Herrmann K, 1976) and are consumed regularly with the human diet. These low molecular weight substances, found in all vascular plants, are phenylbenzo- pyrones (phenylchromones) with an assortment of structures based on a common three-ring nucleus.

Flavonoids are polyphenolic compounds that occur ubiquitously in plant foods. Flavonols and flavones are subclasses of flavonoids (Fig. 1).

Although these compounds may not be vitamins as previously postulated, they may constitute a class of semi-essential food components on the basis of

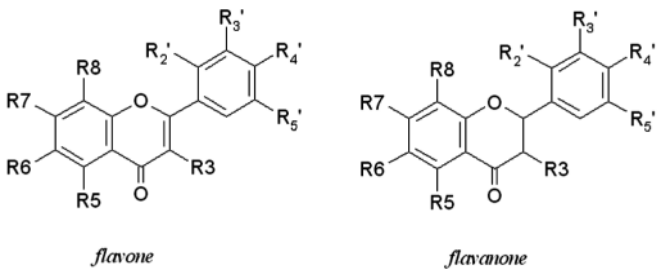


Fig. 1: Structure of Flavonols and Flavones

their ubiquity, their multiple biologic actions and their essential role in insects. Flavonoids have antioxidant and chelating properties and have been shown to modulate various cellular enzymes including kinases, hydroxylases, hydrolases, transferases and oxidoreductases (Kandaswami C. and Middleton E. 1994). Has been suggested and recognized their potential use as antiinflammatory, antiviral, antitumor or antiallergenic agents (Bors W. et al., 1990).

The daily USA diet was estimated to contain approximately 1 g of mixed flavonoids expressed as glycosides (Kuhnau J., 1996). According to Hertog et al. (1992) (Hertog MGL et al., 1992), the average intake of mixed flavonoids was only 23 mg/day based on data from the 1987-88 Dutch National Food Consumption Survey (Hertog MGL, et al., 1993). The flavonoid consumed most was quercetin, and the richest sources of flavonoids consumed in general were tea (48% of total), onions, and apples (Hertog MGL, et al., 1993). The amount of 23 mg/day was mostly flavonols and flavones measured as aglycones. The corresponding amount of daily aglycones consumed in the USA would be about 650 mg/day, since Kuhnau had estimated 1 g/day to be the daily flavonoidglycoside consumption. Although there is a 5-fold difference between the estimates of Kuhnau and Hertog, it should be stressed that recent evidence indicates that flavonoid-glycosides are much more readily absorbed (than the aglycones) by humans (Hollman PC and Katan MB, 1998). Moreover, both the amount and the source could vary appreciably in different countries. For instance, the amount consumed could be considerably higher in the Mediterranean diet, which is rich in olive oil, citrus fruits, and greens. These quantities could provide pharmacologically significant concentrations in body fluids and tissues. Nevertheless, flavonoid dietary intake far exceeds that of vitamin E, a monophenolic antioxidant, and that of β-carotene on a milligram per day basis (Hertog MGL, et al., 1993). A resurgence of interest in traditional Eastern medicine during the past two decades, together with an expanded effort in pharmacognosy, has rekindled interest in the flavonoids and the need to understand their interaction with mammalian cells and tissues.

Flavonoids may have existed in nature for over one billion years and thus have interacted with evolving organisms over the eons. The flavonoids possess some important purposes in nature, having survived in vascular plants throughout evolution. The very long association of plant flavonoids with various animal species and other organisms throughout evolution may account for the extraordinary range of biochemical and pharmacological activities of these chemicals in mammalian and other biological systems.

The four main groups of flavonoids are listed in Table 1, together with the best-known members of each group and the food source in which they are present.

Table 1
Main Groups of Flavonoids: Individual Compounds and Food Sources

Group	Compound	Food sources
Flavones	Apigenin	Apple skins
	Chrysin	Olives
	Kaempferol	Parsley
	Luteolin	Onions
	Myricetin	Berries
	Rutin	Broccoli
	Quercetin	Celery
	Sibelin	Fruit peels
		Cranberries
		Grapes
		Lettuce
Flavanones	Fisetin	Citrus peel
	Hesperetin	
	Narigin	
	Naringenin	
	Taxifolin	
	Citrus fruit	

Of historical importance is the observation that a mixture of two flavonoids called citrin and hesperidin were considered to possess vitamin-like activity (Clemetson CAB, 1989). The term vitamin P was coined to indicate that this material had the property of decreasing capillary permeability (and fragility), prolonging the life of marginally scorbutic guinea pigs, and reducing the signs of hypovitaminosis C in experimental animals. Although so-called vitamin P was shown ultimately not to fulfill the definition of a vitamin and the term was appropriately abandoned, there was nonetheless a strong indication that the flavonoids had potent antioxidant-dependent and vitamin C-sparing activity (Clemetson CAB, 1989).

Moreover, some flavonoids also have anticarcinogenic properties. The flavonoids do not have carcinogenic potential in experimental animals (Aeschbacher H-U., et al., 1982). There is much controversy regarding the purported toxic or even mutagenic properties of quercetin. Formica and Regelson (Formica JV, et al., 1995) gave an interesting overview of the in vitro and vivo studies on quercetin. The early data on toxic side effects are mainly derived from in vitro studies. At a conference of the Federation of American Societies for Experimental Biology in 1984 on mutagenic food flavonoids, carcinogenicity was reported in just 1 of 17 feeding studies conducted in laboratory animals. However, in other long-term studies, no carcinogenicity was found (Zhu BT, et al., 2001). In contrast with the potential mutagenic effects of flavonoids in earlier studies, several more recent reports indicate that flavonoids, including quercetin, seem to be antimutagenic in vivo. A large clinical study by Knekt et al. (Landolfi R, et al., 1984), in which 9959 men and women were followed for 24 y, showed an inverse relation between the intake of flavonoids (e.g., quercetin) and lung cancer. An explanation for these conflicting data is that flavonoids are toxic to cancer cells or to immortalized cells, but are not toxic or are less toxic to normal cells. If this is true, flavonoids might play a role in the prevention of cancer that is worthy of further investigation.

Alcoholism is a prevalent human disorder, and the search for effective remedies continues. For about 2000 years, the Chinese have recognized the antidipsotropic effect of Radix puerariae, an herb used in Chinese traditional medicine for the treatment of alcohol abuse. Keung and Vallee (1993) (Keung WM and Vallee BL, 1993) took advantage of the propensity for alcohol of the Syrian golden hamster to study the effect of extracts of R. puerariae and of daidzin and daidzein, two isoflavones found in the extracts. The isoflavone compounds effectively reduced ethanol consumption in the Syrian golden hamsters by approximately 50%, thus pointing the way to the development of a new class of therapeutic agents for alcoholism. Another briefly reported observation of potentially great significance is the finding of quercetin in bovine retinal tissue. Do ingested flavonoids accumulate in various tissues and modulate their functions? An excellent review of flavonoids in health and disease has been published (Rice-Evans CA,, et al., 1996). Das et al. (1994) (Das A, et al., 1994) conducted a careful structure-system-activity-relationship study of flavonoids with special respect to carcinogenicity, mutagenicity, and cancerpreventing activities. They concluded, in spite of some ongoing controversy, that not only are the "vast majority of flavonoids and isoflavonoids completely innocuous, but may be beneficial in a variety of human disorders".

2. FLAVONOIDS ACTIVITY AND FLAVONOIDS AS SIGNAL MOLECULES

Plant-derived foods that are rich in flavonoids are regularly touted in the popular press for their benefits in ameliorating age-related diseases. A majority of these reports focus on the antioxidant characteristics of flavonoid-rich diets and their enhancement of cardiovascular health. as flavonoids often mimic endogenous mammalian receptor ligands (Virgili et al., 2005) or interfere with the uptake of substrates not found in plants, the applicability of such studies to plant models requires caution. However, studies of flavonoid function in animal cells often provide important insights into their functions as signal molecules in plants. In plants, flavonoids appear to contribute to a general reduction of reactive oxygen species and therefore impact cellular processes sensitive to redox effects. However, flavonoids also have been implicated in more direct interactions with transport and signal transduction pathways. Another is flavonoid modulation of auxin transport as well as localized auxin accumulations observed during nodulation.

At the molecular level, potential targets of flavonoid regulation in plants range from transcription factors and kinases to ATP-binding cassette (ABC) transporters and aminopeptidases. Some of these targets are suggested primarily by similarities between plant and mammalian signaling mechanisms. Other endogenous or exogenous targets, such as receptors, ABC transporters, and hydrolases, have been directly demonstrated in planta or in vivo. Most of these interactions have been shown to be developmentally regulated.

As yet, there is no evidence for direct flavonoid modulation of translation in plants. However, flavonoids have been shown to affect mammalian translation by altering phosphorylation status. Genistein and quercetin were found to inhibit protein synthesis in mouse tumor cells via phosphorylative activation of eIF2-α kinases (Ito et al., 1999), and genistein decreased late viral mRNA translation

via a tyrosine kinase-dependent mechanism (Xi et al., 2005).

There is increasing evidence that specific proteins or groups of proteins exhibit more specific interactions with flavonoids in vivo. Many of these interactions have been shown to depend on the B-ring substitution pattern of the interacting flavonoids (Marko et al., 2004). Flavonols are the most common of these molecules, and their high degree of activity is suggested by the tight developmental and spatial regulation of flavonol synthesis (Murphy et al., 2000; Peer et al., 2004). Studies in animal cell lines have shown that flavonoids alter multiple kinase and phosphatase activities (Harbone and Williams, 2001). Apigenin inhibits protein kinase C (PKC) and MAPK (Kuo and Yang, 1995; Huang et al., 1996). Quercetin is routinely used as an inhibitor of mammalian PIPK, phospholipase A2, phosphodiesterases, and PKC by binding to the catalytic domain (Tammela et al., 2004). Quercetin also inhibits $Ca2^{+}$-dependent and phospholipid-dependent protein kinase activities (Gschwendt et al., 1983). Kaempferol inhibits mammalian monoamine oxidase/peroxidases, of which the IAA oxidase is a family member (Sloley et al., 2000). Kaempferol, quercetin, and genistein inhibit the CDC25A tyrosine phosphatase, a cell cycle-specific protein that is dephosphorylated in M phase (Aligiannis et al., 2001).

3. FLAVONOID ACTIVITY AND TOXICITY

The antiviral activity of flavonoids was shown in a study by Wang et al. (Wang HK et al., 1998). Some of the viruses reported to be affected by flavonoids are herpes simplex virus, respiratory syncytial virus, parainfluenza virus, and adenovirus. For example, some flavonoids work on the intracellular replication of viruses, whereas others inhibit the infectious properties of the viruses. Most studies of the effects on viruses were performed in vitro and little is known about the antiviral effect of flavonoids in vivo. There is some evidence that flavonoids in their glycone form seem to be inhibitory on rotavirus infectivity than are flavonoids in their aglycone form. Because of the worldwide spread of HIV since the 1980s, investigations of the antiviral activity of flavonoids have mainly focused on HIV. Many natural products can inhibit various stages of the replication cycle of the virus. The discovery and development of flavonoids as anti-HIV agents has expanded in the past two decades. Most of these studies focused on the inhibitory activity of reverse transcriptase, or RNA-directed DNA polymerase (Ng TB et al., 1997), but antiintegrase and antiprotease activities were also described. Again, flavonoids have mainly been studied in in vitro experiments; therefore, no clear contribution of flavonoids to the treatment of HIV-infected patients has yet been shown (Vlietinck AJ et al., 1998). The flavonoids are formed in plants and participate in the light-dependent phase of photosynthesis during which they catalyze electron transport (Vlietinck AJ et al., 1992). They are synthesized from the aromatic amino acids, phenylalamine and tyrosine, together with acetate units (Heller W and Forkmann G, 1993). Phenylalamine and tyrosine are converted to cinnamic acid and parahydroxycinnamic acid, respectively, by the action of phenylalamine and tyrosine ammonia lyases. Cinnamic acid (or parahydroxycinnamic acid) condenses with acetate units to form the cinnamoyl structure of the flavonoids (Fries rearrangement). A variety of phenolic acids, such as caffeic acid, ferulic acid, and chlorogenic acid, are cinnamic acid derivatives. There is then alkali-catalyzed condensation of an orthohydroxyacetophenone with a benzaldehyde derivative generating chalcones and flavonones, as well as a similar condensation of an ortho-hydroxyacetophenone with a benzoic acid derivative (acid chloride or anhydride), leading to 2-hydroxyflavanones and flavones (Heller W and Forkmann G, 1993). The synthesis of chalcones and anthocyanidins has been described in detail by Dhar (1994) (Dhar DN, 1994). Biotransformation of flavonoids in the gut can release these cinnamic acid (phenolic acids) derivatives.

Flavonoids are complex and highly evolved molecules with intricate structural variation. In plants, for instance, they generally occur as glycosylated and sulfated derivatives.

On the other side, some epidemiologic studies suggest a cardioprotective role of flavonoids against coronary heart disease. One large clinical study indicated that flavonoids may reduce mortality from coronary heart disease (Hertog MGL et al., 1993). Various cohort studies indicated an inverse association between flavonoid intakes and coronary heart disease mortality. These studies are promising and indicate that flavonoids may be useful food compounds. Flavonoids have received much attention in the literature over the past ten years and a variety of potential beneficial effects have been elucidated. However, most of the research involved

in vitro studies; therefore, it is difficult to draw definite conclusions about the usefulness of flavonoids in the diet. The study of flavonoids is complex because of the heterogeneity of the different molecular structures and the scarcity of data on bioavailability. Furthermore, insufficient methods are available to measure oxidative damage in vivo and the measurement of objective endpoints remains difficult. There is a need to improve analytic techniques to allow collection of more data on absorption and excretion. Data on the long-term consequences of chronic flavonoid ingestion are especially scarce. In conclusion, the in vivo studies that have been performed do give a hopeful picture for the future. Currently, the intake of fruit, vegetables, and beverages (e.g., tea and moderate amounts of red wine) containing flavonoids is recommended, although it is too early to make recommendations on daily flavonoid intakes.

Research on flavonoids in relation to the human diet has mainly focused on the flavonol quercetin, primarily because of early methodologies enabling sensitive detection of this compound in biological fluids and in foods. It has been found that other groups of flavonoids, such as the citrus flavanones, may be more important dietary constituents than the flavonols. The flavanones are major contributors to the total dietary intake of flavonoids in Denmark (Justesen, U., et al., 1998) and in Finland, for example, and may be absorbed to a much higher extent than flavonols, such as quercetin (Zhu BT et al., 2001).

To evaluate the influence of flavonoids in our diet and their usefulness as a marker of certain vegetable or fruit groups, it is crucial to monitor the concentration of the major dietary flavonoids concurrently in human biological samples.

Several different dietary flavonoids are measurable in urine from subjects on their habitual diet, and that the sum of flavonoids excreted in urine is associated with the intake of fruits, berries, and vegetables. Urinary flavonoids may therefore be a valid biomarker for fruits, berries, and vegetables (FBV) intake. This is supported by the positive correlations between urinary flavonoid excretion and habitual FBV intake, the significant differences in flavonoid excretion between high and low FBV diets, and by the positive correlation between the deltas of FBV intake and flavonoid excretion during the dietary intervention.

In the plant kingdom, the flavonoid biosynthetic pathway is ubiquitous and produces a variety of pigmented as well as nonpigmented compounds. Flavonoid compounds have been implicated in several biological processes and some of their functions include the attraction of pollinating agents via pigmentation of floral organs (Huits H.S.M., et al., 1994), pollen tube germination (Mo Y., et al., 1992), protection from UV exposure (14), and defense against insects by acting as insecticides (Wiseman B.R. et al., 1996) and fungal pathogens by acting as phytoalexins (Nicholson, R.L. and Hammerschmidt R., 1992). Flavonoid biosynthesis takes place through the phenylpropanoid pathway, and depending on the genetic constitution of the plant naringenin can have several different fates leading to the formation of flavonoid metabolites that include anthocyanins, flavones and anthocyanidins (Winkel-Shirley B., 2001). Flavonoid pigments have been used as a convenient visible marker in molecular genetic experiments and to study regulation of gene expression. Since the isolation of chalcone synthase, which catalyzes the first committed step of this pathway, efforts have been focused on the isolation of mutants and the cloning of structural genes that are required for the different biosynthetic steps (Winkel-Shirley B., 2001).

A nutraceutical is any nontoxic food extract supplement that has scientifically proven health benefits for both the treatment and prevention of disease. Nutraceuticals may range from isolated nutrients, dietary supplements, and diets to genetically engineered “designer” food, herbal products, and processed products, such as cereals, soups, and beverages. The increasing interest in nutraceuticals reflects the fact that consumers hear about epidemiological studies indicating that a specific diet or component of the diet is associated with a lower risk for a certain disease.

The major active nutraceutical ingredients in plants are flavonoids. The flavonoids are a group of organic molecules ubiquitously distributed in vascular plants. Approximately 2000 individual members of the flavonoids group of compounds have been described. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators. They also appear to be effective at influencing the risk of cancer. Overall, several of these flavonoids appear to be effective anticancer promoters and cancer chemopreventive agents. The presentation in this chapter is designed to provide

the reader the tools to understand the biological and molecular role of plant flavonoids, including their antioxidant and antiproliferative activities and their role in intracellular signaling cascades.

Cancer is the third major cause of mortality, accounting for more than 7 million deaths per year worldwide (Cancer Facts and Figures, 1997). The major goal of cancer research, therefore, has been to achieve an understanding of the processes involved in the induction followed by development of human cancers, which could allow diagnosis, an early detection, and therapy as well as prevention of this disease.

Chemoprevention of cancer, therefore, is a means of cancer control by which the rate of this disease can be prevented totally or slowed or reversed partially or substantially by the administration of one or more naturally occurring or synthetic chemical agents. Fruits, vegetables and common beverages as well as several herbs and plants with diversified pharmacological properties have been shown to be rich sources of microchemicals with the potential to prevent human cancers (Birt D.F., et al., 1996; Morse M.A., et al., 1993). Measuring the effects of these agents in cancer chemoprevention studies in human populations has now become one important objective of experimental cancer research. The potential for inhibiting tumor development in both targeted high-risk and general populations has increased significantly in recent years.

About 30 classes of chemicals with cancer-preventive effects that may have practical implications in reducing cancer incidence in human population have been described (Wattenberg L.W., 1997).

Among these, naturally occurring polyphenolic antioxidants are receiving increasing attention in recent years. Using different long-term experimental tumorigenesis protocols, several studies have demonstrated the cancer-preventive effects of polyphenolic antioxidant. Silymarin is also a polyphenolic flavonoid antioxidant isolated from milk thistle. It is being used clinically in Europe and Asia for the treatment of alcoholic liver diseases. As a therapeutic agent, silymarin is well tolerated and largely free of adverse effects (Vogel G., et al., 1975), so much so that silymarin has also been marketed recently in the United States and Europe as a nutritional supplement by several "nutraceutical companies". Studies on mice, rats, rabbits, and dogs, using different modes of administration, showed that silymarin is nontoxic in acute tests, even at large doses. Similarly, it is nontoxic in subchronic and chronic tests and does not show any side effects; there is no known LD50 for silymarin in laboratory animals (Wagner H., et al., 1969; Ely H., 1989). Several studies in rodents have shown that silymarin protects against hepatotoxicity induced by allylalcohol, carbon tetrachloride, galactosamine, phalloidin and thioacetamide. Mechanistic studies have shown that silymarin is a strong antioxidant that is capable of scavenging both free radicals and reactive oxygen species in rodents and in cell cultures and that it results in a significant increase in cellular antioxidant defense machinery by ameliorating the deleterious effects of free radical reactions. Carcinogenesis in mouse skin and other animal tumor bioassay systems and, possibly, in humans is a stepwise process of at least three distinct stages: initiation, promotion, and progression. For more than 50 years, mouse skin has been used as a conventional model to study the mechanism of carcinogenesis and modulation of sequential steps involved in this process (Agarwal R., et al., 1991; Yuspa S.H., 1994). In fact, this is the oldest experimental model to demonstrate the multistep process of carcinogenesis and to define the cellular, biochemical, and molecular mechanisms associated with each stage (Yuspa S.H., 1994). The mouse skin carcinogenesis model, which provides a conceptual framework to study the carcinogenesis process, has also been used extensively to: 1. assess whether chemical and/or physical agents carry a carcinogenic hazard to humans and 2. evaluate the cancer-chemopreventive effects of different agents and define the mechanism involved with their protective effects (Agarwal R., et al., 1991; Yuspa S.H., 1994).

Using the skin carcinogenesis model, Santosh K. Katiyar and al found that a naturally occurring polyphenolic flavonoid antioxidant, silymarin, is an exceptionally high anti-tumor promoting agent. (Santosh K. et al., 1999).

The fate of orally and parenterally administered flavonoids in mammals and the significance of biliary excretion was reviewed by Griffiths and Barrow in 1972. Since then, progress in understanding flavonoid phar- macokinetics has been slow. Published studies of flavonoid metabolism are not extensive, and were reviewed again recently (Hollman PC and Katan MB, 1998). Such studies are essential to enhance our understanding of the possible importance of flavonoids in human health

and disease. Considerable information is available regarding the metabolism of flavonoids in animals and to a very limited extent in humans. Ring scission occurs under the influence of intestinal microorganisms, which also account for the subsequent demethylation and dehydroxylation of the resulting phenolic acids (cinnamic acid derivatives and simple phenols).

Intestinal bacteria also possess glycosidases capable of cleaving sugar residues from flavonoid glycosides. Such glycosidases do not appear to exist in mammalian tissues. Flavonoids can undergo oxidation and reduction reactions, as well as methylation, glucuronidation, and sulfation in animal species. Oral administration (83 mg/kg) resulted in rapid absorption, metabolism, and excretion of the flavonoid within 24 h. Eleven metabolites were detected in urine. No quercetin could be found in plasma after oral administration of up to 4 g in humans (Shali NA et al., 1991). Hepatic metabolism of quercetin and catechin by isolated perfused rat liver has been demonstrated in studies by Shah et al. (1986) (Shah GM and Bhattacharya RK, 1986). The flavonoids were converted into sulfated and/or glucuronidated metabolites, which were excreted in the bile. Recent improvements in analytical techniques have made possible the determination of baicalein and baicalin (the glycoside of baicalein) in rat plasma by high pressure liquid chromatography with electrochemical detection (Wakui Y., et al., 1992). Oral administration of these flavonoids to rats led to readily measurable concentrations of the compound in plasma (100–10,000 ng/ml).

The most important information derived from recent studies is the fact that most flavonoids, except catechins, exist in nature as glycosides. Moreover, at least quercetin glucosides were absorbed better than the aglycone quercetin-β-glucoside (Hollman PC and Katan MB, 1998). Consequently, the amount of flavonoid glycosides consumed is a better indication than the amount of aglycones, thus raising the lower level estimated for the flavonoid aglycones. Finally, supplementation of the diet should more appropriately use flavonoid glycosides instead of aglycones.

Adverse reactions to flavonoids in humans appear to be rare. Studies of Salama and Mueller-Eckhardt (Salama A and Mueller-Eckhardt, 1987) indicated that catechin and its metabolites can bind tightly to erythrocyte membranes and that this generates new antigenic sites with consequent development of autoantibodies presumed to be the cause of hemolytic anemia in six patients who had taken the catechin. The hemolytic anemia disappeared after discontinuation of catechin ingestion although the subjects continued to ingest cross-reactive dietary flavonoids. Some flavonoids are capable of quinone formation, a familiar pathway leading to contact sensitization. The flavonoids are not potent contact allergens and are not distinguished as contact sensitizers in the dermatologic literature, even though essentially all human beings have daily physical contact with flavonoid containing foods and plants.

The flavonoids appear to be remarkably safe nutrients with a wide range of biochemical and pharmacologic activities that strongly suggest their possible role as health-promoting, disease-preventing dietary supplements.

A large number of studies have emphasized the potential health-promoting and disease- preventing effects of fruits and vegetables in the diet. The beneficial effects of fruits and vegetables have frequently been attributed to ascorbic acid and the carotenoids present in these foods. However, as stated elsewhere, fruits and vegetables contain a multitude of flavonoids and related phenolic compounds that also act as natural antioxidants. Flavonoids can function as:

- metal chelators and reducing agents,
- scavengers of ROS,
- chain-breaking antioxidants,
- quenchers of the formation of singlet oxygen,
- protectors of ascorbic acid; conversely, ascorbic acid can protect flavonoids against oxidative degradation.

In many of the studies reported, it is not certain whether flavonoids inhibit the formation of ROS or scavenge them. It is obvious that flavonoids react with OH and, therefore, can be very important chainbreaking antioxidants. They could also play an important role in conserving tocopherols in biological membranes. Ascorbic acid (ascorbic acid is a universal component of plant cells) and flavonoids coexist in many plants and thus the two may be consumed together in the diet. A large body of literature has accumulated concerning the interactions of flavonoids with ascorbic acid in biological systems. Several flavonoids serve as antioxidants for ascorbic acid.

Flavonoids have been considered to function as antioxidants and UV light filters in higher plants. This antioxidant activity was related to their protection against ascorbic acid oxidation. The protection of ascorbic acid by flavonoids could have important biological implications, as emphasized by Hughes and Wilson (Hughes RE and Wilson HK, 1997).

The role of flavonoids in extraorganismal plant signaling has been described extensively, but the role of flavonoids in intra- and intercellular signaling is not as well documented. Flavonoid signaling between organisms may involve visual cues, as in pollinator attractants, as well as molecular cues. Flavonoids are bioactive molecules with specific and nonspecific effects on intraand extraorganismal plant signaling mechanisms. However intraorganismal flavonoid signaling is probably a by-product of the evolution of plant signaling and trafficking mechanisms in an environment where flavonoids are present for purposes of extraorganismal signaling and defense and a role in initial protection from oxidative stress. Recently developed molecular biological tools and high throughput metabolic profiling technologies provide new opportunities to identify specific and nonspecific sites of flavonoid regulation.

4. QSAR ANALYSIS

In predictive toxicology, we exploit the toxicological knowledge about a set of chemical compounds in order to predict the degree of activity of other compounds (Hansch, C., Fujita, T., 1964). More specifically, we mathematically model the relationship between specific properties of training compounds (i.e. compounds for which the degree of activity is known) and their toxicological activity and apply the model to query compounds (i.e. compounds for which the degree of activity is not known) to obtain predicted activities.

The process of model-building is called (Quantitative) Structure Activity Relationship ((Q)SAR). SARs are models based on structural features, and QSARs rely on quantitative (frequently physicochemical) properties. The most general mathematical form of a (Q)SAR is:

$$\text{Activity} = f\ (\text{physicochemical and/or structural features})$$

Multilinear models have been in use since a long time. As linear equations, they are easy to use and relatively straightforward to interpret. For n instances they are defined as the coefficients that minimize the error on a system of n linear equations (Pavan M., et al., 2006; Putz, M.V., et al., 2009):

$$y_i = b_1 x_{i1} + b_2 x_{i2} + \ldots + b_m x_{im} + d \quad i \in \{1, \ldots, n\}$$

or in a more compact notation,

$$y = (\langle X, b \rangle + d)$$

where $\langle \cdot, \cdot \rangle$ denotes the normal dot product and b and d are the coefficients to learn. Multilinear models assume linear relationships between features and activities, therefore the expressiveness is limited and the model will perform poorly if these conditions are met (Pavan M., et al., 2006). The prediction $f(x_q)$ is obtained by (Putz M.V., et al., 2009):

$$f(x_q) = (\langle x_q, b \rangle + d)$$

Relationships were developed to correlate a structural parameter (i.e., lipophilicity) with activity. In some cases, mono-parametric relationship correlating structure and activity was adequate. The form of the equation is:

$$A = \log\left(\frac{1}{C}\right) = a + b \log P$$

where C is the molar concentration of compound that produces a standard response (e.g., LD_{50}, ED_{50}).

Log P in particular was found to be important in many QSAR models. The octanol/water partition coefficient (log P) constitutes a quantitative, and easily accessible, hydrophobicity measurement (G.E., Kellog, et al., 2001; K.M., Biswas, et al., 2003). P is defined as the ratio of the equilibrium concentration of a substance dissolved in a two-phase system, formed by two immiscible solvents:

$$P_{o/w} = \frac{C_{octanol}}{C_{water}}$$

The relationship between log P and some biological responses was often inverse parabolic, in which a maximum in the biological response occurred at some optimum log P value. The explanation for this relationship was that it described the partitioning of drug molecules into biological membranes.

$$A = \log\left(\frac{1}{C}\right) = a + b \log P + c\ (\log P)^2$$

Most acute toxicity studies aim to determine the median lethal dose (LD_{50}) of the chemical. The LD_{50} is defined as a statistically derived expression of a single dose of a chemical that can be expected to kill 50% of animals in the experimental group (Putz, M.V., et al., 2008; Putz, M.V., et al., 2009). The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

As a working example, in the Table 2 some data for the series of flavonoids (50% lethal toxicity dose EC_{50} (μM) were presented from literature data (Zhang S., et al., 2005), among the employed activity $A = -\log_{10}(EC_{50})$ and structural parameters as hydrophobicity LogP and polarizability POL (Å^3). The molecules included in the "Gaussian or Normal" set have their index marked with G while those considered in the "Non-Gaussian or Non-normal" set were marked as N and leave out for the first statistical analysis (the so called Non-Gaussian-Leave-Out screening method).

Table 2
The Flavonoids of Fig. 1 Arranged upon their Ascending Observed Activities, Defined as $A = -\log_{10}(EC_{50}$ [μM]) (Zhang S., et al., 2005), Along the Associate Computed Structural Parameters Like the Hydrophobicity (LogP), Electronic Cloud Polarizability (POL) and the Ground State Configurationally Optimized Total Energy (ETOT)

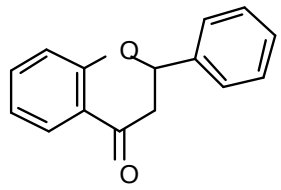
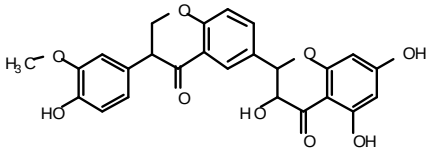
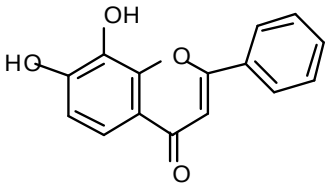
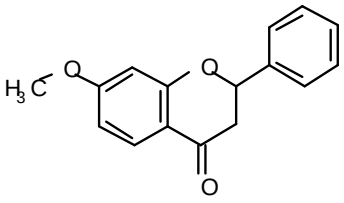
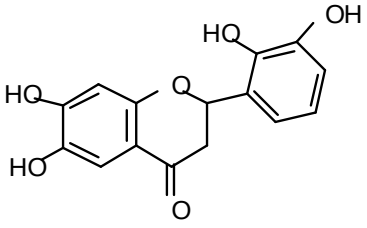
G/NG	Molecular name	Structure	Activity A	Structural parameters		
				LogP	POL (Å^3)	E_{TOT} (kcal/mol)
(G)	Flavanone		4.6	2.84	25.55	–62849.3125
(NG)	Silybin		3.74	2.03	45.68	–146625.1875
(G)	7, 8-Dihydroxyflavone		4.7	1.75	26.63	–76982.1328
(NG)	7-Methoxyflavanone		4.79	2.59	28.02	–73823.8046
(G)	6, 2', 3' –7-Hydroxyflavanone		4.85	1.70	28.10	–92422.6640

Table Contd.

Table 2 Contd.

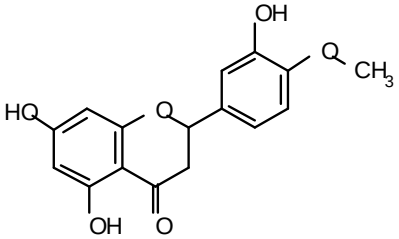
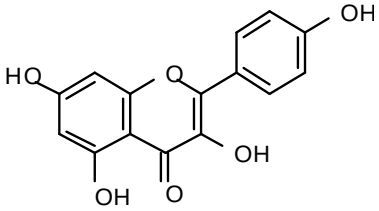
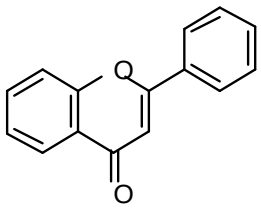
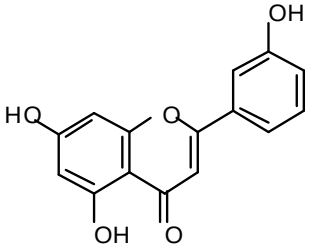
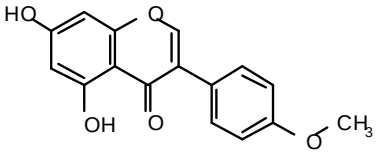
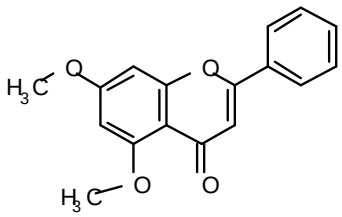
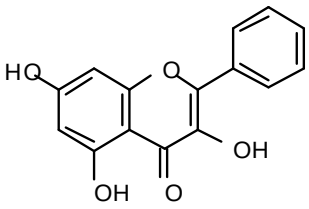
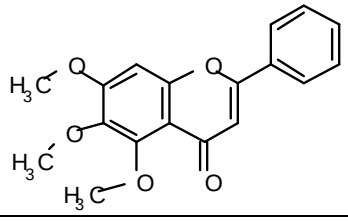
(NG)	Hesperetin		4.91	1.73	29.93	−96003.9921
(NG)	Kaempferol		5.22	0.56	27.90	−91770.5859
(G)	Flavone		5.4	2.32	25.36	−62196.3437
(G)	Apigenin		5.78	1.46	27.27	−84379.8593
(NG)	Biochanin A		5.79	1.53	29.10	−87961.2812
(NG)	5, 7-Dimethoxyflavone		5.85	1.81	30.30	−84139.4687
(NG)	Galangin		5.92	0.85	27.27	−84376.8359
(G)	5, 6, 7-Trimethoxy-flavone		5.96	1.56	32.77	−94976.1875

Table Contd.

Table 2 Contd.

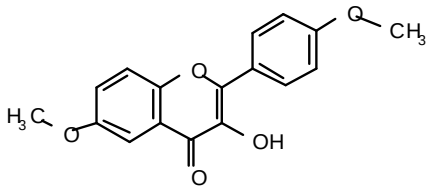
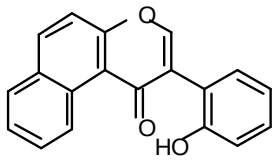
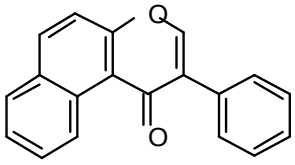
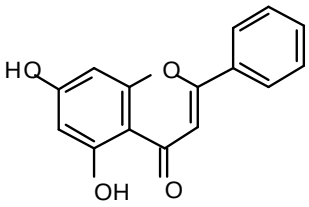
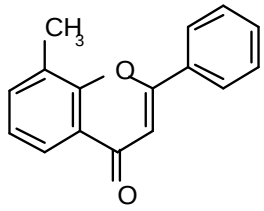
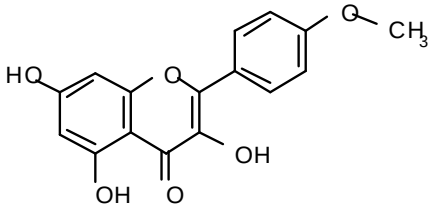
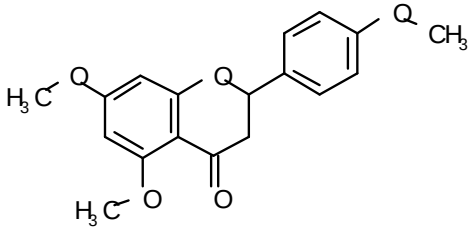
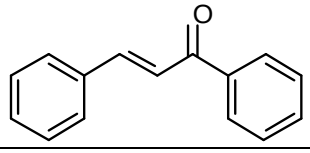
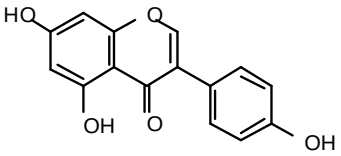
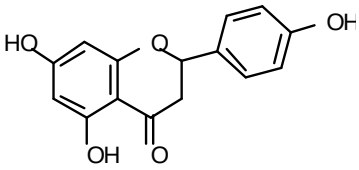
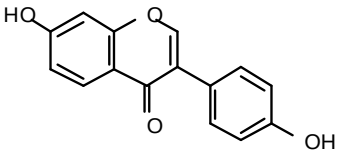
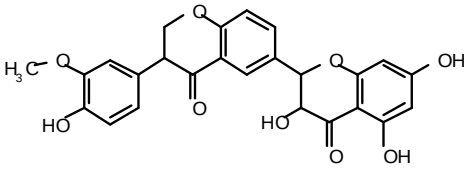
(G)	6, 4'-Dimethoxy-3-hydroxy-flavone		6.35	0.41	31.13	−92162.7187
(G)	2'-Hydroxy-α-naphto-flavone		7.03	3.07	33.26	−82027.8359
(G)	7, 8-Benzoflavone		7.14	3.35	32.63	−74634.5234
(G)	Chrysin		6.41	1.75	26.63	−76986.1171
(G)	8-Methylflavone		6.21	2.79	27.19	−65789.9218
(G)	Kaempferide		5.99	0.60	29.74	−95351.3984
(G)	4'−5, 7-Trimethoxy-flavanone		5.25	2.08	32.96	−95768.9062
(G)	Chalcone		4.93	3.68	25.49	−55450.1093

Table Contd.

Table 2 Contd.

(G)	Genistein		4.83	1.50	27.27	−84380.7578
(G)	Naringenin		4.49	1.99	27.46	−85032.9218
(G)	Daidzein		4.24	1.78	26.63	−76984.7109
(NG)	Silybin		3.74	2.03	45.68	−146625.1875

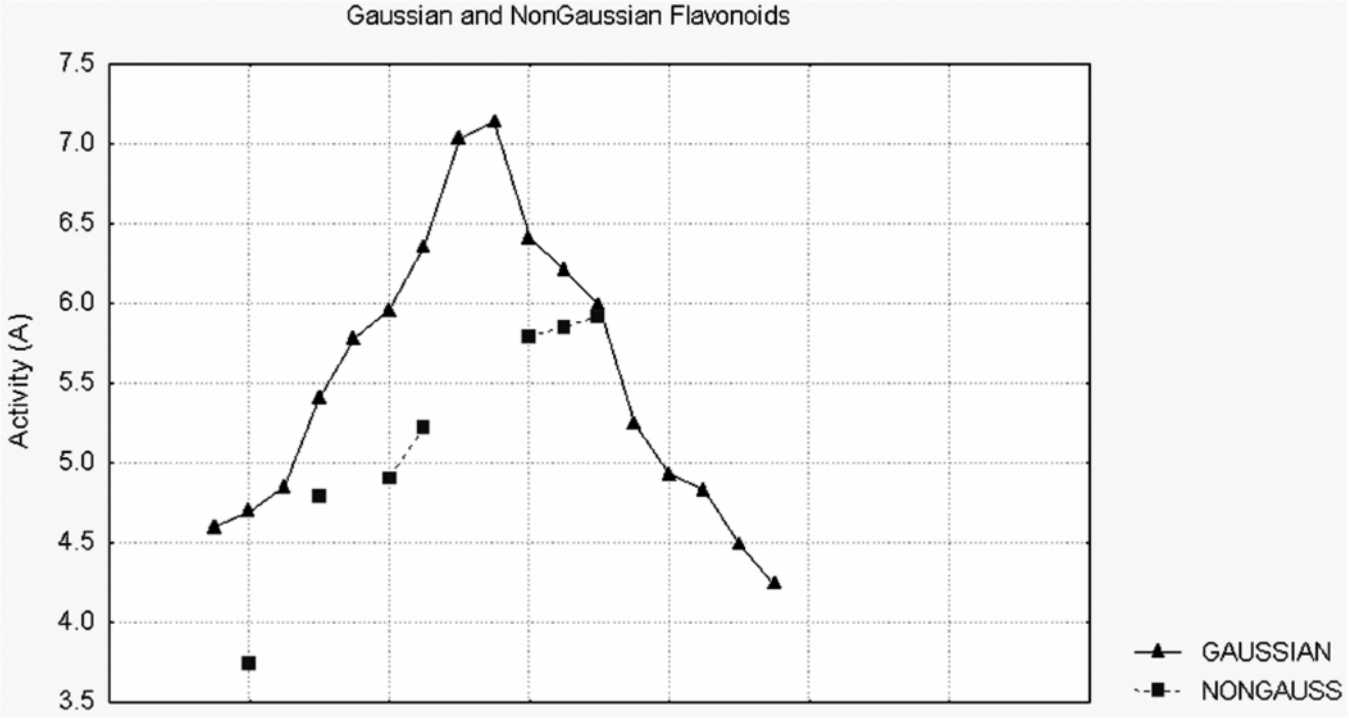


Fig. 2: The Plot of the Flavonoids' EC_{50} (activity) Toxicities of Table 2

We obtained some data (structure activity relationships) for all possible lipophilicity correlation models considered from the data in Table 1 together with the statistical (simple correlation factor, standard error of estimation SEE). The results are in the Table 3 presented for Gaussian molecules only.

In the QSAR models given above, N is the number of data points, R is correlation coefficient and SEE is standard error of estimate between the variances of calculated and observed activities.

From the Table 3 we obtain useful information about the structure parameters in correlation with hydrophobicity and biological activity. Going to the

Table 3: Structure Activity Relationships for the "Gaussian-G" Flavonoids Molecules of Table 2

Model Equation	R	SEE	N
LogP = 1.9174 – 0.0004 Pol	0.0204	0.8679	17
A = 5.6431 + 0.0178 LogP – 0.0083 Pol	0.0445	0.9052	17
A = 5.3983 + 0.0179 LogP	0.0176	0.8851	17
$A = 6.6024 – 1.4558\ LogP + 0.3718\ (LogP)^2$	0.3984	0.8367	17
$A = 5.6645 – 1.1393\ LogP + 0.0250\ (LogP)^2 + 0.3934\ Pol + 0.00012\ E_{TOT}$	0.7089	0.6719	17

activity-hydrophobicity analysis, for the series flavonoids molecules we identify such low R value for the simple relationship between toxicity and first order hydrophobicity indicating practically no correlation or chemo-bio-influence. Instead, it significantly grows for relationship indicating the toxicity description in terms of the descriptors (logP) and $(logP)^2$ meaning that the toxicity (EC_{50}) of this flavonoids could be described best by a parabolic model including the hydrophobicity.

Even more, with inclusion of the other electronical structural information as polarizability and the total energy one records the minimum of SEE and the maximum of R telling that the model A = f (Pol, logP, $(logP)^2$, ETOT) is predicted as the most reliable; it leads with idea that the ionic or electrostatic character of the interaction primary influences the hydrophobicity towards the final stabilization by the steric (energy-optimum configuration) effect. The graphical representation of the computed-predicted activity by this model is in Fig. 3 represented.

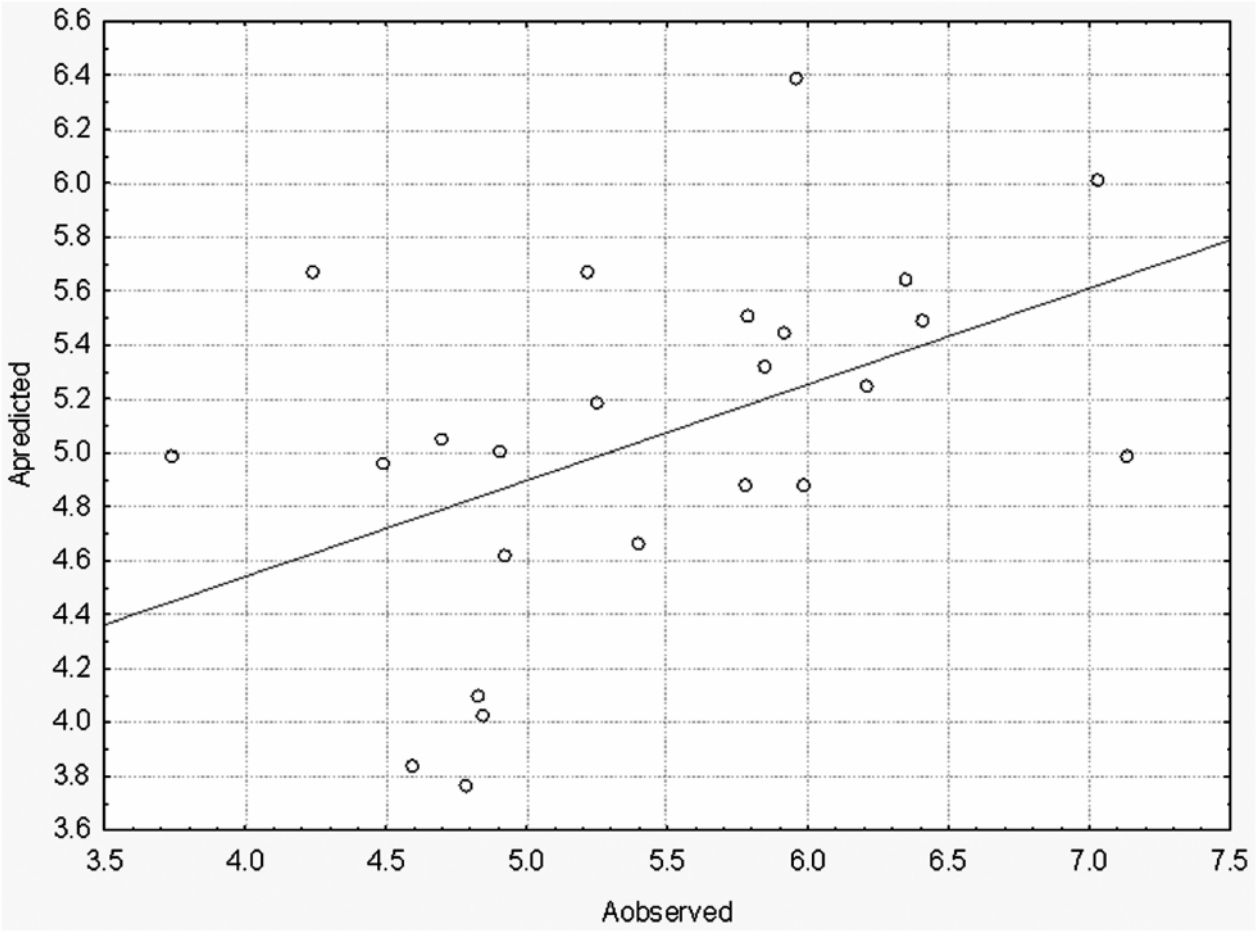


Fig. 3: Relationship of the Predicted Toxicity Values with the Observed Values for the QSAR Model of Table 3 for the Quadratic Hidrophobicity Dependence Along the Electronic Structure Factors (Polarizability and the Total Energy) Included

5. CONCLUSION

While reviewing themain flavonoid molecular features and use in present days one may study their quantitative impact on biological activity in environment by using the QSAR method as a representation of target (ligan-receptor) theory. Simple screening method of gaussian vs. non-gaussian record of activity has been advanced and used to select the molcules in the congeneric series "ready" to be studied for structure-activity multilinear relationship. For a sample series of flavonoids molecules it has been found that there is an important "transition" phase in causing bioactivity: that is when the chemical transport process is no longer described only by first order hidrophobicity logP, that eventually cause an ever increment in activity and lowers correlation with chemcial ligands, while achiving the minimum and thus reaching the "optimum valey" of correlation when the second order of hydrophobicity $(logP)^2$ considered; the QSAR model may be then even mor eimproved by adding the other specific electronic structural informationinto correlation such as the polarizability an dthe total energy, thsu ensuring the quest of "local minimum" of residues in chemo-bio interaction while providing the highest correlation coeffcient with this approach.

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