Bioactive Chromone Derivatives – Structural Diversity

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Abstract: Chromones (1-benzopyran-4-ones) and chromone derivatives are naturally occurring compounds ubiquitously found in the plant kingdom, and therefore present in representative amounts in a normal human diet. These phytochemicals possess a wide spectrum of biological activities – such as anti-inflammatory, antifungal, antimicrobial, antiviral, antitumour and anticancer – mainly due to their well-recognised antioxidant properties, which stem from their ability to neutralise active forms of oxygen and to cut off free radical processes.

Since oxidative stress is known to be the basis for numerous severe pathologies, from cardiovascular and neurodegenerative disorders to cancer, the development of effective antioxidant agents from natural origin (namely dietary constituents) has been the object of vigorous research in the last decade, in view of establishing novel chemopreventive strategies against such diseases which are nowadays the main cause of death worldwide. In fact, the chromone moiety is an important element of pharmacophores of many biologically active molecules displaying diverse medicinal applications.

These potential health benefits arising from the antioxidant activity of chromone derivatives are ruled by strict structure-activity/structure-property relationships, which, apart from determining their biological action, modulate their systemic distribution and bioavailability in sites of oxidation within the cell.

The present work aims at reviewing the main reported studies on the cytoprotective and anticancer activities of chromone derivatives, with particular emphasis on the effect of their structural features and conformational behaviour on activity, which is the basis of a tailored design of novel chromone-based antioxidants for chemopreventive and chemotherapeutic use.

Keywords: Chromones, chemopreventive, chemotherapeutic, anticancer, structure-activity relationships (SAR).

1. INTRODUCTION

Modern lifestyle and diet has led to an increased exposure to free radicals, which is the cause of a higher susceptibility to disease. In fact, these oxidative stress conditions – disruption of the homeostatic balance between free radical generation and the production of naturally occurring antioxidants (e.g. glutathione and regulatory enzymes such as superoxide dismutase, catalase and peroxidases) – are well-known to be directly linked to a number of disorders due to oxidative damage in numerous cell targets (DNA, lipids, proteins). This finally result in severe diseases such as liver toxicity, cardiovascular and neurodegenerative (e.g. Alzheimer and Parkinson’s) pathologies, and cancer (Scheme 1).

These disorders are largely preventable through changes in diet, lifestyle and environmental conditions [1]. Hence, controlling or restoring the homeostatic oxidative balance is of the utmost relevance in the medical field.

Antioxidant defence mechanisms, however, are species dependent and heavily influenced by nutrition, since important antioxidants, such as ascorbic acid and α-tocopherol, cannot be synthesised by humans and must therefore be obtained exogenously [2]. Consequently, research focused on new antioxidants has been steadily developing in the last decade within the dietary and pharmacological fields, as evidenced by the number of new antioxidant products targeted for both the food industry and the cosmetics market, as well as for medicinal applications. In fact, the most amazing and effective tools for assisting human oxidative defence and fight disease may be growing in our own backyard [1,3-15].

Phytochemicals are defined as chemicals produced by plants, mostly concentrated in fruits, vegetables, wine, tea and cocoa, which display significant antioxidant and chelating properties. They are not considered as essential nutrients (such as proteins, carbohydrates, fats, minerals or vitamins), but are recognised to possess health-promoting properties since they have been progressively associated to biochemical defence mechanisms against bacteria, fungi, viruses and damage to cell structures, especially DNA. The three major classes of plant chemicals have been identified as phenolic derivatives, terpenoids and alkaloids [16]. The phenolic constituents, which are secondary metabolites, are the largest group and comprise anthocyanins, anthochlor, benzofurans, chromones (1-benzopyran-4-ones, Fig. (1)), coumarins, flavones and isoflavones (Fig. (2)), flavonones and flavonol, lignans, phenolic acids (hydroxybenzoic and hydroxycinnamic) and ketones, phenylpropanoids, quinonoids, stilbenoids, tannins and xanthones [17]. These polyphenols also present a

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Scheme 1. Oxidative stress and its major biomedical consequences.

Fig. (1). Schematic representation of the chromone structure. (The atom numbering is included).

Fig. (2). Schematic representation of chromones (4H-benzyopyran-4-one, (A)), flavones (2-phenyl-1,4-benzopyrone, (B)) and isoflavones (3-phenyl-1,4-benzopyrone, (C)).
distinctive ability to form inter-molecular interactions, either with each other (yielding dimeric species), or with other molecules such as biogenic polyamines (e.g. putrescine, spermidine or spermine) giving rise to a large variety of phenolic amides abundant in plants.

Mainly due to their well-recognised antioxidant properties – as a result of their marked tendency to transfer electrons, chelate ferrous ions, or scavenge reactive radicals – these naturally occurring heterocyclic compounds (comprising oxygen) are known to display pharmacologically relevant properties, namely antibacterial, antifungal, antiviral, anti-spasmodic, anti-inflammatory, anti-HIV and anticancer [18-22]. In fact, protection against cancer as well as towards several age-related chronic diseases, provided by many common food constituents, has been observed in multiple epidemiological studies, and generally ascribed to non-nutritive dietary antioxidants such as chromone analogues. Consequently, vigorous research (basic investigation and studies in both animal models and humans) has been carried out in order to understand and improve these potential health-beneficial agents. Prevention of cancer and cardiovascular distress through dietary intervention has therefore become an essential subject of research in the pharmacological and medicinal chemistry fields.

Although the use of plants for medicinal uses by indigenous people has been carried out for centuries, it was not before the 1980s and 1990s that the studies on phytochemicals entered the academic and industrial laboratories, aiming at their development for use as bioactive substances displaying specific health benefits – as preventive agents (nutraceuticals) or even as medicines – highlighting the importance of prevention, mainly when it became clear that therapy does not always succeed (e.g. due to drug resistance mechanisms) [23]. Therefore, strategies have been envisaged to modify dietary habits and complement the normal day-to-day diet with specific phytochemicals, as potential sources of antioxidants for phyto-pharmaceutical applications and functional food ingredients. Nevertheless, the complexity of the interactions between phytochemicals and other dietary components requires a thorough understanding of their mechanisms of action, which, in turn, relies on a detailed knowledge of the structural features and conformational preferences of these systems.

This manuscript is intended as a minireview comprising the main studies published in the last decade on the chemopreventive and chemotherapeutic properties of chromone derivatives, particularly against cancer, emphasizing the importance of structural and conformational factors on this biological activity. These structure-activity relationships (SAR’s) are the basis for the development of lead compounds aiming at a rational design of effective, chromone-based, preventive and/or anticancer agents.

2. CHEMOPREVENTION VERSUS CHEMOTHERAPY

It is widely established that exogenous antioxidants are needed for reducing the cumulative effects of oxidative damage over a life span, given the incomplete efficiency of our endogenous defence systems, their progressive breakdown with age and the existence of several factors responsible for ROS generation at the wrong time and place – cigarette smoke, air pollutants, UV radiation, fatty acid diet, inflammation, ischemia, etc. Thus, apart from the well-established dietary antioxidants vitamins C, E, and A, and carotenoids, phytochemicals are gaining increasing importance as potential protective antioxidants.

There is presently compelling evidence that polyphenolic derivatives have an important role in chemoprevention and even chemotherapy against free radical mediated diseases such as cancer. They have been reported to interfere with the initiation, promotion and progression stages of the carcinogenesis process, highlighting their importance as potential preventive and/or therapeutic natural agents. In fact, the intake of these compounds (e.g. chrome derivatives), namely through the diet, has been inversely related to the risk of developing various common neoplasms, such as lung, digestive tract and hormone-related cancers. Prostate cancer is a meaningful example: while hormone-refractory prostate cancer (HRPC) is a significant cause of death among men in the United States of America [24], individuals residing in Asia show a quite lower HRPC incidence when compared to caucasian males. In turn, asian men who move to and live in the USA, adopting a western lifestyle, have HRPC rates indistinguishable from caucasian males. These findings [25] suggest that asian diets contain ingredients that might protect against the development of this type of cancer, probably owing to a functional synergy between bioactive substances such as epigallocatechin gallate, genistein and quercetin.

Rossi and collaborators [26-29] have evaluated the relationship between flavonoid (2-phenyl-chromone, Fig. 2)) intake and the appearance of several types of human cancer, having established chemoprotective effects towards colorectal, pharyngeal, laryngeal, oral and ovarian cancer risk for this type of chromone-based compounds. Regarding prostate cancer, however, the results obtained by these researchers for this particular chrome analogue do not support a protective effect [30], which reflects selectivity, another relevant feature of the anticancer protective activity of this kind of systems. Additionally, dietary polyphenols were shown to possess beneficial cardiovascular effects [31,32].

These results add to the feasibility of attacking disease with an effective weapon – prevention – by developing a diet-based combinatorial approach, based on dietary polyphenols. Whereas chemotherapy is designed to destroy cancer after its appearance, chemoprevention aims at avoiding or at least delaying the onset of the neoplasm. The last decades of research in the field established prevention as the approach of choice in the struggle against cancer, as this pathology is much easier to prevent than to treat, and the former can be accomplished with a high degree of success through consumption of certain fruits and vegetables in the daily diet. Since cancer is a multifactorial disease, numerous pathways and molecular sites are to be considered as the targets of chemoprevention: apoptosis induction and downregulation of antiapoptotic proteins; suppression of growth factor expression; interference with intracellular signalling cascades; and downregulation of angiogenesis, among others. Phytochemicals, including chromone derivatives, have been determined as pharmacologically safe compounds able to modulate these specific molecular targets [33-35].
Chemoprevention is therefore generally accepted as the main benefit of natural and synthetic chromone constituents. Nonetheless, research on this type of systems, within a pharmacological and medicinal chemistry outlook, has progressively shown that they are also capable of exerting therapeutic effects and even of reversing chemoresistance [36,37], thus growing to be promising agents for use in novel chemotherapeutic strategies, mostly in combination with conventional therapies [38,39]. However, the mechanisms underlying their activity are not completely characterised, and many features still remain to be elucidated at a molecular level in order to clearly understand their mode of action and interference with cellular targets.

3. CHROMONES AS BIOACTIVE AGENTS

Chromones, a group of natural compounds comprising the 4H-benzopyran-4-one skeleton (Fig. (1)) present in several species of higher plants, are responsible for significant biological functions at nontoxic concentrations in living organisms. They display a marked antioxidant capacity [29, 40-49], through two main antioxidant pathways: 1) direct reaction with free radicals; 2) chelation of metal ions such as Fe(II) and Cu(II), involved in free radical-generating reactions (e.g. production of reactive oxygen species). Also, natural and dietary phenolic compounds (e.g. green tea polyphenols, soy isoflavones) have shown to be effective in suppressing invasiveness of cancer cells [50]. This is known to be a fundamental process in tumour progression and metastasis, intimately related to cell growth, cell adhesion, cell migration and proteolytic degradation of tissue barriers, which are mediated by specific intracellular signalling mechanisms. This inhibition of the invasive behaviour of cancer cells by dietary compounds can be considered as an adjuvant or combination anticancer therapy. Chromones and their structural analogues have therefore motivated a great interest within the medicinal chemistry field, the chromone moiety being the essential component of pharmacophores of a large number of bioactive molecules.

The term antioxidant has lately been redefined in a broader sense, to identify any substance that directly scavenges reactive oxygen species (ROS) (including deleterious cations such as Fe(II) or Cu(II)) or indirectly acts to upregulate antioxidant defences or inhibit ROS production. Chromones, in particular, are able to scavenge a wide range of ROS and can inhibit lipid peroxidation [42]. Although H-abstraction contributes most to this hydroxyl-radical-scavenging reactivity, electron transfer and the stability of the corresponding phenoxy radicals resulting from H-abstraction also play an important role in this process. Free radical scavenging occurs by rapid donation of a hydrogen atom to the radical, and is therefore primarily attributed to the high reactivities of hydroxyl substituents that participate in this reaction:

\[
\text{C-OH} + \text{R}' \rightarrow \text{C-O}^* + \text{RH}
\]

The radical scavenging activity of chromone derivatives obeys to strict conformational requirements, and is therefore determined by their molecular structure and substitution pattern (mainly OH substitution), which is reflected in the availability of phenolic hydrogens and in the degree of stabilisation of the resulting phenoxy radical, either through H-bonding or by electron delocalisation [51,52].

Numerous studies are to be found, mostly in the last decade, on chromones and their analogues (e.g. flavonoids) as biologically active agents. Chromone derivatives, which are easily absorbed and metabolised, have been reported to display such significant activities as antimicrobial [53] and antifungal [47,54], anti-inflammatory [47,55-59], immunostimulatory [60], HIV-inhibitory [61,62], antimutagenic [63], antiproliferative [12,28,60,64-70], inductive of cell cycle arrest [44] and apoptosis [71-73], angiogenesis inhibition, and even reverse of multidrug resistance [12,39]. They were also found to act as neuroprotective [74] and anti-psychotic [75], and to exhibit antiplatelet aggregation effects [56,76], therapeutic efficacy against arthritis [74] and hypolipidemic activity [78], being also reported to function as selective sigma receptor ligands [79].

Different substitution patterns of the chromone skeleton can lead to considerable changes in their conformational behaviour and consequently their properties and function. Two-substituted chromones, in particular, exhibit a remarkable variety of biological activities, such as antiallergic or antineoplastic against several human cancer cells (e.g. leukaemia). Furthermore, chromones often disturb mitochondrial bioenergetics and enzymatic activities (e.g. succinate dehydrogenase, succinate cytochrome c reductase, and cytochrome c oxidase), in a dose-dependent way [80].

Sesquiterpene chromone derivatives (Fig. (3)) were reported to inhibit nitric oxide radical production [57,81]. In fact, NO is known to play a central role in inflammatory and immune reactions, excessive production leading to tissue damage (e.g. rheumatoid arthritis). Phenylethylchromones (with different hydroxyl and methoxyl substitutions [82]), in turn, have shown remarkable effects, namely at the central nervous system – 5-hydroxy-2-(2-phenylethyl)chromone, for instance, acts as a neuroprotector against glutamate-induced neurotoxicity [74,81]. Eugenitol (2,8-dimethyl-5,7-dihydroxy-chromone) and eugenitnine (2,6-dimethyl-5-hydroxy-7-methoxy-chromone), which are important constituents of clove oil, were reported to behave as carcinogenesis inhibitors thanks to their remarkable antioxidant capacity. Noduliprevenone, extracted from marine fungus, is a heterodimeric chromanone reported to act as a cancer chemopreventive agent via modulation of the xenobiotic metabolism [83].

**Fig. (3).** Schematic representation of some sesquiterpene chromones (Adapted from [57]).
A new chromone derivative isolated from a marine fungus, 2-(hydroxymethyl)-8-methoxy-3-methyl-4H-chromen-4-one (chromanone A), showed to act as a promising inhibitor of several carcinogen metabolising enzymes [70]. Moreover, it displays a powerful and specific radical scavenging activity against hydroxyl radicals (more than peroxyl radicals), which is responsible for its protective effect towards oxidation-induced DNA damage in cells. Hence, chromanone A is a potential tumour anti-initiating agent.

Baicalein, a trihydroxylated flavonoid derivative, was found to be a potent and selective human lipoxigenase (LO) inhibitor [84], through direct binding of its catecholic moiety to the iron centre at the enzyme’s active site. This chromone derivative may therefore become a promising chemopreventive/chemotherapeutic agent, since lipoxigenase is implicated in pathologies such as asthma, immune disorders and several types of cancer.

Tangeretin (5,6,7,8,4´-pentamethoxy flavone) and nobiletin (5,6,7,8,3´,4´-hexamethoxy-flavone) (Fig. (4) (A) and (B)) are polymethoxylated flavonoids concentrated in the peel of citrus fruits, that are among the most effective antioxidants with promising pharmacological properties, such as antibacterial (particularly the 2-phosphoforms) and antiproliferative effect towards several human carcinomas inducing cell death through G1 arrest and DNA fragmentation [97]. While the number and position of the hydroxyl groups in the A-ring is undoubtedly relevant for the scavenging effect of these molecules, the styryl moiety was found to be responsible for an enhanced molecular stabilisation, therefore increasing the compound’s antiradical activity. A synthetic 2-styrylchromone, (3´-allyl-4´,5,7-trimethoxy-2-styrylchromone) was reported to disturb mitochondrial energetics in rats, leading to cell death through the induction of procaspases and a strong decrease of the available ATP [80]. A deleterious effect on the integrity of the mitochondrial membrane was also verified, leading to a respiratory activity depression.

Fig. (4). Schematic representation of polymethoxylated chromone derivatives: tangeretin (A), nobiletin (B) and zapotin (C).

Fig. (5). Schematic representation of styrylchromones.

Phosphorus-containing chromones [98], in turn, constitute a novel group of compounds with interesting biological properties, such as antibacterial (particularly the 2-phosphorylated derivatives) and anticancer towards several tumour cell lines.

Glycosylated chromones (O- or C-glycosylated), either natural such as aloesin (extracted from various Aloe species) [58,59,99] (Fig. (6)) and baicalin (a baicalein O-glucuronide [100]), or synthetic such as hyperimone [101] (Fig. (7)), were shown to inhibit tyrosinase activity, improve insulin
sensitivity [102], exert hepatoprotective activity or even behave as cytotoxic agents [99]. This latter feature is of the utmost importance, since liver toxicity caused by chemicals and drugs has been long recognised as a serious toxicological problem, urging for the search of effective hepatoprotective agents obtained from natural sources. Also, due to the role of tyrosinase in melanin production, aloesin and its analogues are often used in cosmetics as inhibitors of hyperpigmentation induced by UV radiation [103]. Uncinoside, an O-glycosylated chromone, was showed to have antiviral activity against respiratory and parainfluenza virus [104] – both uncinoside A (5-hydroxy-2,6,8-trimethylchromone-7-O-β-D-glucopyranoside) and uncinoside B (5-acetoxy-2,6,8-trimethyl-chromone-7-O-β-D-glucopyranoside). A C-glucosyl-chromone extracted from Aloe barbadensis (8-[C-β-d-[2-O-(E)-cinnamoyl]glucopyranosyl]-2-[{(R)-2-hydroxypropyl]-7-methoxy-5-methylchromone}) has been reported to have significant topical anti-inflammatory properties, comparable to those of hydrocortisone, without any reduction in thymus weight (as opposed to the classical hydrocortisone treatment) [105]. In fact, A. Vera has long been known to be a source of biologically relevant compounds with a wide range of health beneficial properties, namely glycosylated chromones [106].

![Fig. (6). Schematic representation of aloesin.](image)

![Fig. (7). Schematic representation of hyperimone A (A) and hyperimone B (B). (Adapted from [101]).](image)

Furthermore, chromones and chromone derivatives are presently being used in adjuvant and/or combination therapy, since the efficiency of classical anticancer drugs has been verified to be improved by co-administration of these polyphenols. This has been observed for breast cancer chemotherapy with paclitaxel, for instance, cotreatment with chromone having led to a modulation of the drug’s transport and thus to a higher efficiency of chemotherapy in paclitaxel-resistant tumours [107].

In addition to these well known biological and medical properties of chromones and analogues, their complexes with metal ions have recently been recognised to be more effective than the ligands alone [19], which has opened new and promising routes in drug development. Although this is out of the scope of the present review, it should be mentioned that this kind of complexes have been successfully used in a range of pathologies such as diabetes mellitus, bacterial infections, certain types of cancer and neurodegenerative diseases (e.g. Huntington’s disorder).

Apart from the recognised antiproliferative properties of chromone derivatives, clinical evidence indicates that cancer patients given phenolic antioxidants regularly exhibited higher tolerance and decreased chemotherapy side effects, and that they live longer and with a higher quality of life [108].

In the light of this broad biological activity, considerable attention has been devoted in the last two decades to the isolation of chromone derivatives from natural sources, and to research regarding their chemistry and biochemical function. Numerous chromone-based phytochemicals have been purified from plant extracts or synthesised de novo, their properties being determined by the different substitution pattern of the chromone skeleton. In recent years, due to increasing public interest in healthy lifestyles and disease prevention and treatment, dietary supplements and nutraceuticals containing high doses of this type of phenolic derivatives have been introduced to the market [109-111]. In addition, some have even entered clinical trials, namely the synthetic flavone flavopiridol (5,7-dihydroxy-8-(4-N-methyl-2-hydroxypropyl)-6-chloroflavone hydrochloride, Fig. (8)), which is the first cyclin-dependent kinase inhibitor to be tested in humans, both in Phase II single-agent trials and Phase I combination trials (with paclitaxel and cisplatin, where a synergistic effect has been observed) [112-114]. In fact, cell cycle regulatory proteins such as cyclins and cyclin-dependent kinases (cdks) are potential molecular targets, since their functions are tightly controlled in normal cells but not in tumour cells. In fact, the preclinical studies on the synthetic flavopiridol evidence its capacity to induce programmed cell death, promote differentiation, inhibit angiogenic processes and modulate transcriptional events [113,114], as well as to enhance radiosensitivity in certain human cancers (by inhibiting repair of the radiation damage) [115].

![Fig. (8). Schematic representation of flavopiridol.](image)

Accordingly, interpretation of this wide biological activity at a molecular level is urgent, aiming at the development of chromone-based compounds with promising pharmacological properties, either chemopreventive or chemotherapeutic against pathologies associated with oxidative stress, such as cancer and neurodegenerative disorders. This requires the establishment of accurate structure-activity relation-
ships, starting by the rational design of a lead compound to be structurally modified and screened as to both cytostatic/cytotoxic and preventive capacities. This structure-based drug design relies on an accurate knowledge of the structural parameters and conformational preferences of the polyphenolic systems.

4. THE STRUCTURAL VARIABLE

Although the chromone moiety seems to be essential for most of the biological activity reported for this group of compounds, this has also been shown to be strictly dependent on other structural parameters, from the nature, number and position of the substituent groups to their relative orientation [116,117]. In fact, even a minor change in one of these factors can induce a considerable variation of the compound’s properties and therefore of its biological function. Conformational preferences such as flexibility, formation of hydrogen bonds – either intra- or intermolecular – and planar or skewed relative orientations of the substituent groups linked to the chromone skeleton determine the properties of the systems. The possibility of occurrence of hydrogen close contacts, for instance, can lead to a quite large stabilisation and therefore to a marked preference of a particular structure as opposed to other likely configurations (e.g. chromone-3-carboxylic acid versus chromone-2-carboxylic acid, Fig. (9) [N.F.L. Machado and M.P.M. Marques, unpublished results]).

![Fig. (9). Schematic representation of chromone-2-carboxylic acid (A) and chromone-3-carboxylic acid (B).](image)

Additionally, the biological activity of these molecules is determined by their capacity to give rise to adducts with proteins, enzymes and biological receptors. Formation of these adducts in vivo is affected by the three-dimensional chromone structure and by the electronic distribution within the molecule – areas of highest electronegativity tend to appear at the 5-hydroxyl and 7-hydroxyl positions of the A-ring, as well as at the various hydroxylation sites on the B-ring (Fig. (1)).

In view of this preventive and therapeutic potential of chromone-derivatives, many researchers have therefore tried to elucidate possible structure-activity relationships that might lead to new drug discovery. Numerous studies are to be found in the literature reporting the synthesis and biologic screening of a series of such compounds, comprising different chemical entities added to a fixed chromone moiety. These structural modifications are then related to the measured activity and interpreted in the light of biochemical and pharmacological data obtained for the systems tested. Actually, while antioxidant activity relies on the presence of hydroxyl groups, other substituents were verified to affect this capacity, or to relate to distinct biological effects (Scheme 2).

![Scheme 2. Main structure-activity relationships (SAR’s) established for chromone derivatives. (DTC – dithiocarbamate).](image)

Samee and coworkers [118] investigated the effect of structural modifications on the antioxidant activity of several chromones, having verified that it relies on the OH substitution in the aromatic A ring (mainly 8-OH substitution), and that the presence of a substituting benzoyl group in ring B (leading to an expanded conjugation system) significantly improved the radical scavenging capacity.

Huang and collaborators [44] described the synthesis and antitumour activities against various human solid tumours of a series of heterocyclic thioether chrome derivatives, determining the effect of a change in the heterocyclic moiety on their antiproliferative and cytotoxic capacities. One of the compounds tested (Fig. (10)) revealed a quite good activity against the mammary line MDA-MB-435S, with an IC_{50} equal to 17.2 μM, similar to that of 5-Fluorouracil (14.5 μM). In fact, numerous studies indicate that the presence of an heterocyclic thioether or a cyclic tertiary amine group will benefit the antitumour activity of chromone-based compounds [119-126].

![Fig. (10). Schematic representation of an heterocyclic thioether chromone derivative which showed a significant activity against the mammary human cancer line MDA-MB-435S. (Adapted from [44]).](image)

The potential antipsychotic effect of several 2-[(4-benzyl-piperazinyl)methyl] chromones, namely their binding ability to sigma sites, was investigated [75]. It was verified that the introduction of a substituent in the chromone moiety, such as the replacement of methylenes by carbonyl groups, or of benzyl by aryl rings, led to a dramatic decrease in the affinity for these sites. In fact, among the most hopeful compounds which were found to act as potent and selective sigma ligands, the one containing a 4-methoxyphenyl substituted pyperazine moiety (Fig. (11)) [75] showed to be a promising candidate for the treatment of psychosis.
In view of the well-known properties of dithiocarbamates (DTC) as antioxidants, fungicides, antivirals and even anti-proliferative agents [127-130], several chromone analogues comprising diverse dithiocarbamate moieties were also synthesised. These were screened as to their in vitro growth-inhibiting capacity towards human tumour cells [43], some of them having shown significant dose-dependent effects and a broad-spectrum of activity. For these series of compounds, bearing the same DTC moiety at either positions 2 or 3 (Fig. (12)), it was verified that the antitumor activity was significantly improved by the introduction of a 6-Cl substituent in the chromone skeleton (as opposed to a chloride in the 7-position), while bulky DTC moieties were shown to be unfavourable. Two of these derivatives – 3-chloro-4-oxo-4H-chromen-2-yl)methyl piperidine-1-carbodithioate (Fig. (13) (A)) and 6-chloro-4-oxo-4H-chromen-3-yl)methyl piperidine-1-carbodithioate (Fig. (13) (B)) – evidenced a noteworthy dose-dependent effect against human mammary adenocarcinoma (MDA-MB-435S) and colon carcinoma (SW-480), through cell cycle arrest (at G2/M) and induction of apoptosis. The latter, in particular, was identified as the most promising candidate as a novel anticancer agent, since it exhibited a broad spectrum of activity, with IC50 values under 1.0 μM for all the cell lines tested (six distinct human carcinomas). The localisation of the DTC group was found to be determinant for activity, since this was drastically reduced when DTC was shifted from position 3 to 6 in the chromone rings. Also, the flexibility of the overall compound is a critical factor, since it rules the interplay between the chromone derivative and the biological target. In fact, removal of the methylene group linking the dithiocarbamate and the chromone B ring led to a marked activity decrease.

Despite the large number of experimental studies on the wide biological functions of chromone derivatives, there is still very little quantitative understanding about the relationships between their structure and conformational preferences, and the reported activity and potential beneficial effect on human health. Actually, the available data (Scheme 2) still lacks a systematic analysis aiming at establishing reliable and useful structure-activity relationships (SAR’s and QSAR’s [131]).

Theoretical calculations, at different levels (namely semi-empirical methods and quantum mechanical approaches such as Hartree-Foch and Density Functional Theory (DFT)), have been carried out for this kind of systems. These allowed to determine the main physical and electronic properties (descriptors) of relevance for antioxidant capacity, namely structural parameters and conformational preferences leading to an optimised biological activity. Several Physicochemical parameters have been determined for numerous phenolic compounds, in an attempt to elucidate the quantitative structure-activity relationships ruling their biological function [52,132-137]. This parameters include, among others, charge, number and relative position of the substituent hydroxyl groups, bond dissociation enthalpy, heat of formation, ionisation potential, electron affinity, electronegativity, electrophilic index, energies of the lowest unoccupied molecular orbital (LUMO) of the radical and of the highest occupied molecular orbital (HOMO) of the parent compound, and partition coefficient.

Such SAR and QSAR studies allowed to estimate the redox potentials and antioxidant activities and have been performed for naturally occurring chromones [138-142], while similar analysis on synthetic chromone derivatives have only recently appeared in the literature [118,138,139,
Several dedicated algorithms have been developed for improving the correlation between predicted and experimental activities for this kind of systems. One of these approaches is the MOLMAP (Molecular Maps of Atom-level Properties), which encode local aspects of a chemical structure exclusively based on physicochemical properties, and can be interpreted as a fingerprint of the molecule’s reactivity [135].

A 3D-QSAR analysis was devoted to a series of synthetic hydroxychromones, for which the DPPH free radical scavenging activity was predicted by theoretical methods [145]. 7-hydroxy, 8-hydroxy and 7,8-dihydroxychromones (Fig. (14)) were evaluated, allowing to conclude that electron-negative substituents on the benzoyl ring and electropositive groups on the phenyl ring play an important role for antioxidant activity, since they may help radical stabilisation throughout the chromone skeleton. In turn, high molecular weight and bulky substituents near position 5 and the carbonyl moiety are disfavoured, as the steric hindrance may disrupt the planarity between ring A and the carbonyl of the chromone nucleus, therefore affecting radical delocalisation [145].

Furthermore, QSAR models of the compound’s effects in biological systems – enzymatic structures, cells and organisms – have recently been attempted [145,146], in order to attain an accurate prediction of the chemopreventive and possible chemotherapeutic activity of the molecules under study. Although this is essential for a rational design of new anti-inflammatory and anticancer chromosome-based agents, it is not a straightforward task, since this type of compounds operate through a diverse array of antioxidant and non-antioxidant mechanisms.

The SAR-type studies carried out to this date on chromone derivatives allowed to identify specific structural components as requisites for radical scavenging, chelation and antioxidant activity. In fact, this was found to be mainly governed by the number, pKₐ values and arrangement of the hydroxyl groups in the chromone ring system, as expected, since these are the groups responsible for the molecule’s reductive capacity through the formation of a stable radical [51,52,147,148]. Hence, a good correlation has been obtained between antioxidant and radical scavenging capacities and the hydroxyl bond strength, electron-donating ability (ionisation potential estimated by the HOMO energy), enthalpy of single electron transfer and spin distribution of the phenoxyl radicals after H-abstraction [52,138]. A double bond and a carbonyl function in the oxygen-containing heterocycle were verified to enhance activity by affording a more stable chromone radical through conjugation and electron delocalisation [147]. In turn, a decrease in the radical scavenging activity was detected upon methylation, mainly when it occurred at the B-ring [51,147,149]. Apart from these key factors, the lipophilic vs hydrophilic character is of the utmost importance in order to ensure both an efficient transport and a high bioavailability [147]. In this regard, the presence of a certain number of hydrophobic moieties has been recognised as beneficial, within a tight equilibrium between hydrophobic and hydrophilic groups. Methoxy groups, for instance (e.g. in tangeretin and nobiletin, Fig. (4)), increase lipophilicity and membrane partitioning, although they introduce unfavorable steric effects [46]. In fact, polymethoxylated chromones and flavones display a significantly low polarity and assume planar structures, which influences their permeabilities to biological membranes, metabolic fates and receptor-binding features.

Fig. (14). Schematic representation of 7-hydroxy- (A) and 8-hydroxychromones (B), as well as 7,8-dihydroxychromones displaying high radical scavenging activity (C to E) (Adapted from [118]).
These properties, in turn, play critical roles in the molecules’ mode of action.

The presence or absence of an unsaturated 2–3 bond (Fig. (1)) in conjugation with a 4-oxo function are also relevant factors for activity. Most of the reported studies support that chromones lacking one or both of these features are less potent antioxidants than those with both elements. In fact, 2–3 unsaturation and a 4-carbonyl group have been correlated to a higher antioxidant capacity and to lower IC₅₀ values in microssomal systems as compared to similar compounds with a saturated heterocycle. In summary, antioxidant potency appears to be enhanced by the presence of both 3-OH and 5-OH groups, in combination with a 2–3 double bond and a 4-carbonyl function, which are structural features that favour the phenoxyl radical stability [52].

7-hydroxychromones, in particular, are known to have a specially high antioxidant activity. Additionally, the relative position of the hydroxyl substituents and the interactions they can establish seem to be more important than the number of these groups. In fact, it was long verified that radical scavenging and metal chelating abilities (e.g. towards iron and cooper) require the presence of at least two vicinal OH’s [42], in a catechol-like arrangement (in fact catechol (1,2-hydroxybenzene) is a potent scavenger whereas phenol (hydroxybenzene) is not).

5. CONCLUSIONS AND FUTURE TRENDS

A regular intake of antioxidant compounds present in food – such as chromones and flavonoids – has long been recognised to be an important health-protecting factor. Accordingly, prevention of cancer through dietary intervention, as opposed to therapy after cancer growth, has been the object of increasing research in the last few years.

Two decades of SAR studies have allowed a basic understanding of the absorption, pharmacokinetics, and metabolism of chromone-derived compounds as a function of their structural and conformational preferences. Furthermore, metabolic changes, which include hydroxylation, O-methylation, cleavage of the heterocycle, deglycosylation, and scission of polymeric species into monomeric units, can not be disregarded, since activity can be either lost or enhanced upon metabolism.

However, despite the unquestionable potential of this family of polyphenolic compounds as health-beneficial agents, supporting a positive role in human nutrition and disease prevention, additional and careful studies are crucial, namely focusing on their possible prooxidant role in the presence of some divalent ions (e.g. Cu(II)). In fact, reports of in vitro cytotoxicity, mutagenicity and proapoptotic effect related to some chromone derivatives raise obvious concerns, since many of these compounds are nowadays marketed as nutraceuticals (e.g. concentrated extracts of propolis, pine bark, green tea leaves, soy isoflavones and grape seeds). This prooxidant activity is thought to be closely related to the total number of hydroxyl groups.

Thus, some of the structural characteristics that optimise antioxidant capacity may also intensify oxidative stress and consequent damage to both functional and structural cellular molecules. Nevertheless, features favouring radical stability and therefore antioxidant activity (such as 3-OH and 5-OH, or 2–3 insaturation) may modulate these possible adverse oxidative effects. Moreover, glycosylation and methylation of OH groups attenuate the prooxidant behaviour of chromones-based compounds. In addition, while the presence of divalent iron or cooper may accelerate prooxidant effects in vivo, chelation of these ions by the chromone analogues may theoretically revert this process. Besides, copper-initiated prooxidant reactions may not be significant in vivo, where the copper ion is largely sequestered, except maybe when certain metal overload diseases occur.

In addition to the structural and physico-chemical features of this type of phytochemicals, their absorption, pharmacokinetics, biotransformation and relative activities of the corresponding metabolites are critical determinants of their biological effects in organisms. Therefore, further investigation is justified in order to elucidate these factors and extend structure-activity relationships to preventive and therapeutic nutritional strategies. In due course, this line of study should lead to dietary recommendations regarding fruits, vegetables and other plant foods in the light of their health benefits, mainly targeting the aging population and individuals with an increased risk of cardiovascular distress, severe inflammatory conditions or cancer. Ultimately, the design of novel polyphenol-based chemopreventive and/or chemotherapeutic agents against oxidation-induced disorders is envisaged, with an emphasis on the development of new targeted therapies aimed at inducing cytostatic (rather than cytotoxic) effects.

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