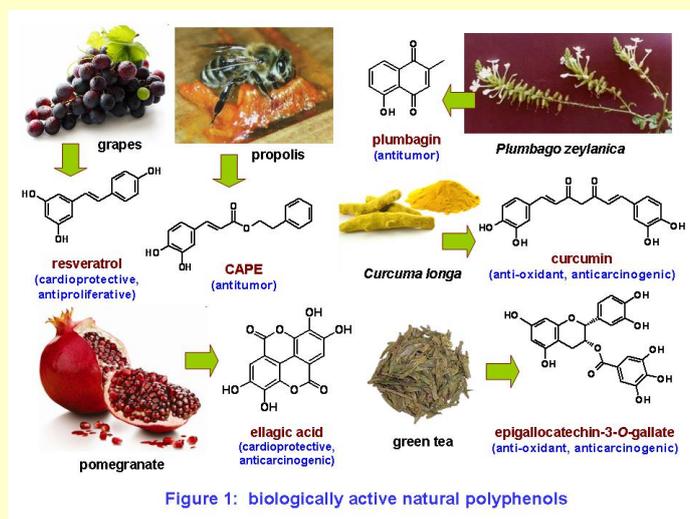


CHEMISTRY OF BIOACTIVE NATURAL PRODUCTS

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Our Chemistry is close to Nature: we study bioactive natural products and in recent years our group has devoted the main part of its research activity to the synthesis of analogues of biomolecules, in particular polyphenols (Figure 1) and nucleic acids. We carry out 'biomimetic' reactions, working also with enzymes, to obtaining compounds potentially useful as anticancer drugs. We also working on projects for a sustainable development, studying the exploitation of agro-industrial biomasses (such as almond crop, wine-making and biofuels waste by-products) to obtain useful products, to be employed as antioxidant for food or cosmetic applications or as adjuvants for pharmaceutical use.

Keywords: natural products; polyphenols; biomasses; synthesis; oligonucleotides.

1. Synthesis of analogues of natural products as potential antitumor agents.

The attention to natural products as 'lead compounds' in drug discovery has been renewed recently. In particular, an authoritative report on *Journal of Natural Products* has highlighted that the majority of the currently employed antitumor drugs is of natural origin, or has been obtained through modification of a natural precursor, or has to be considered 'mimetic' of natural products on the basis of its mechanism of action (D.J. Newman and G. M. Cragg, *J. Nat. Prod.* 70, 462-477, 2007). In addition, it has been observed that many polyphenols, frequently present in foods or beverages, show cancer chemopreventive properties, associated with low or absent cytotoxicity, and may be useful 'adjuvants' in cancer therapy. For these reasons, our group in recent years has devoted the main part of its research activity to synthetic analogues of natural products with enhanced antiproliferative or antiangiogenic properties; angiogenesis inhibition (controlled by VEGF, Vascular Endothelial Growth Factor) is a new tool in cancer chemotherapy: without new blood vessels, in fact, a carcinoma cannot grow beyond a very small size, nor can it metastasise to distant organs. We have focused our attention on the optimization of the properties of selected natural polyphenols; in fact, many antitumor diet polyphenols, such as the well known stilbenoid **resveratrol**, found in grape and red wine (see Figure 1), have a low bioavailability, being easily converted to metabolic intermediates. Thus, employing chemical and enzymatic methods, we have recently synthesized a number of resveratrol analogues (including *E* and *Z* isomers) some of them showing optimized metabolic stability and higher antiproliferative activity than the natural model; among these, *Z*-3,5,4'-

trimethoxystilbene showed potent antiproliferative activity against DU-145, LNCaP (prostate), KB (mouth epidermoid) and SW480 (colon) cancer cells; other *cis* methoxylated stilbenoids proved to be potently active against SW480 cells and a docking study revealed their binding affinity with the colchicine site of tubulin in analogy with combretastatin A. Interestingly, the *E*-2-hydroxy-3,5,4'-trimethoxystilbene proved to be also an antiangiogenic agent with VEGF-inhibitory properties. Further studies on methylated/acetylated resveratrol analogues showed that other synthetic stilbenoids attenuated steroidogenesis and modulated mitochondrial function in Leydig cells. More recently, a tetramethoxy- and a pentamethoxystilbene have showed promising drug resistance inhibition properties (ABCG2 inhibition). Further synthetic efforts have been addressed to the preparation of hydrosoluble resveratrol analogues, and the 3-*O*-phosphorylresveratrol, a potential prodrug of the natural stilbenoid, resulted more active than resveratrol itself against DU-145 cells, and showed interesting DNA-interacting properties, which were even stronger for the related 4'-*O*-phosphorylresveratrol. Our current synthetic projects on resveratrol derivatives are addressed to two different targets: a) conjugated resveratrol analogues, incorporating a colesteryl pendant, to be synthesized through phosphoramidites intermediates; b) analogues of viniferins, a group of natural stilbenoid dimers with antitumor properties, to be obtained through enzymatic oxidative coupling of stilbenoid monomers.

In current literature, the term '**lignans**' is commonly employed to indicate a large family of widespread natural products, biosynthetically originated by oxidative coupling of two phenylpropanoid (C₆C₃) units, which display an impressive structural diversity and a comparable variety of biological activities. In particular, some neolignans have been reported as antimitotic, antiangiogenic and pro-apoptotic agents. Thus, lignans and related compounds are an attractive target for chemical synthesis or modification. We employed biomimetic dimerization reactions carried out through a radical phenolic oxidative coupling of natural precursors, to obtain 'unnatural' products by a mechanism mimicking the 'natural' biosynthetic process. We have recently carried out the biomimetic oxidative coupling of caffeic esters, namely **CAPE** (Caffeic Acid Phenethyl Ester, a well-known component of propolis, see Figure 1) and **methyl caffeate**. The reaction afforded with good yields (72% for CAPE dimerization) unusual, fluorescent **benzo[*k*]xanthene lignans** (see Figure 2) as main products.

We carried out also a mechanistic study of this oxidative coupling reactions, and applied this methodology to synthesize the natural benzoxanthene lignans rufescidride and mongolicumin.

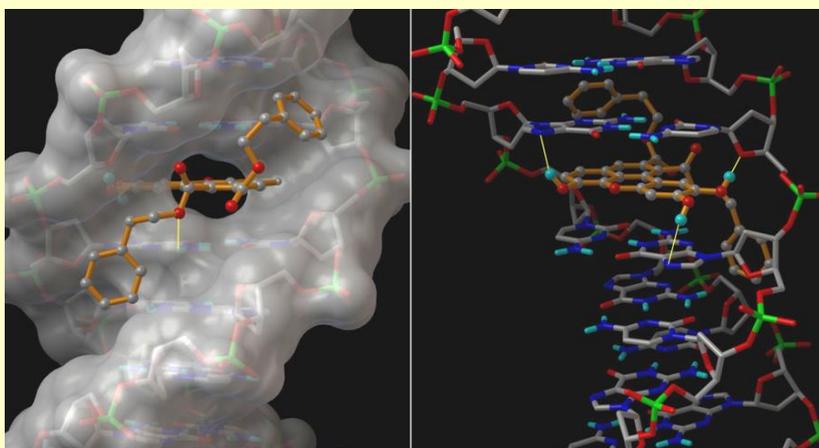


Figure 3: 3D interactions of benzo[*k,l*]xanthene lignan with DNA

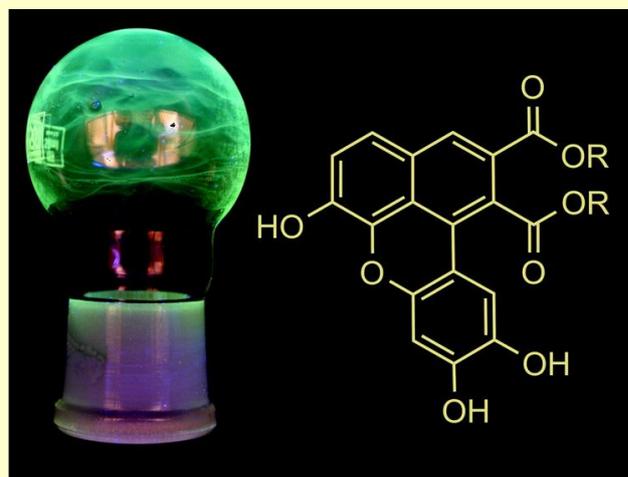


Figure 2: reversed round-bottomed flask containing a solid sample of the CAPE-derived benzo[*k,l*]xanthene lignan (R = phenyl ethyl)

480 (colon carcinoma) and HepG2 (hepatoblastoma) cancer cell lines. A small panel of compounds

Benzo[*k*]xanthene lignans are very rare both among natural products and synthetic analogues and consequently are almost unexplored with regard to their biological properties and possible pharmacological applications. Thus, we studied their interaction with DNA through NMR based approach and molecular docking, paralleled by evaluation of their antiproliferative activity towards SW

was used to obtain preliminary SAR data. The results reveal that the planar chromophoric moiety of the benzoxanthene lignans is intercalated between two DNA base pairs and the flexible appendages are collocated along the grooves of nucleic acid and make contacts with the external deoxyribose/backbone (Figure 3). Docking studies, in agreement with NMR data, show that the bulky phenylethyl groups, giving wider Van der Waals contacts with the minor groove, improve the binding affinity, as highlighted by the higher calculated inhibition constants and the higher observed antiproliferative activity. Some of these lignans are currently under evaluation for inhibition of Hypoxia Inducible Factor 1 α , a mediator of the cell response to the reduced oxygen supply in many solid hypoxic tumors, which are more resistant to radiation and chemotherapeutic drugs than their normoxic counterparts. Further natural-derived compounds related to polyphenols will be synthesized in the next future, and their antiproliferative activity, vascularisation inhibitory properties and interaction with DNA will be evaluated.

2. Synthesis of oligonucleotides conjugated to natural lipids

Great interest is found in the literature regarding to the synthesis of oligonucleotides conjugated to various lipophilic moieties. But, until a few years ago, it was not possible to prepare phosphatidyloligonucleotides owing to an actual difficulty encountered in a direct attachment of the phosphatidyl group to oligonucleotides elongated on the solid phase by standard phosphoramidite chemistry procedures. Some current synthetic opportunities, available for the preparation of oligonucleotides bearing residual base-labile side groups, has allowed us recently to design a synthetic path for obtaining **5'-O-phosphatidyloligonucleotides**.

So, by applying this synthetic route we have prepared some phosphatidyloligonucleotides having all the antisense sequence against the VEGF gene (Figure 4), but differing each other in their phosphatidyl moiety consisting of different fatty acids; stearoyl, palmitoyl and myristoyl residues being employed for this purpose. The spectral properties and the ability of these phosphatidyloligonucleotides to interact with lipid monolayers to give stable duplexes with the target sequence have been studied. Further evaluation of biological activity of the newly synthesized phosphatidyl antisense molecules is in progress.

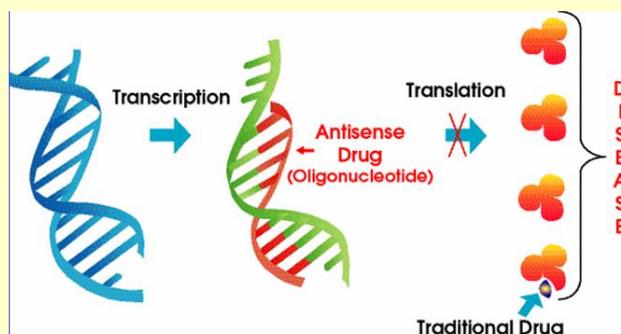


Figure 4: mechanism of action of antisense oligonucleotides

2. Agro-industrial biomasses as a source of antioxidative and antitumor agents

Agro-industrial biomasses are a source of potential antitumor compounds; in fact, these materials are rich of antioxidative constituents. Polyphenol-enriched fractions with enhanced antioxidant activity may be employed in food, cosmetic or pharmaceutical industry. In addition, due to the relationship between antioxidant and pro-apoptotic activity these components may be useful also as adjuvants for cancer therapy. We have recently identified by HPLC-UV-MS and/or chromatographic isolation compounds with antioxidative or antiproliferative properties in wine-making or almond crop by-products; in particular from grape pomace we obtained antioxidative fractions, whereas



Figure 5: *Brassica napus* oilseed cake from biodiesel production

bioguided chromatographic isolation from grape stems afforded antiproliferative constituents, among them **resveratrol**; from almond hulls we obtained a number of bioactive constituents and in particular **betulinic acid** showed potent MCF-7 cell growth inhibitory activity. This methodology will be applied to further biomasses from *Vitis vinifera* or *Olea europea*. In addition we are carrying out studies on oilseed cakes (Figure 5) from biofuel production (*Brassica* spp. and *Helianthus annuus*.) The aim of this work is to obtain further polyphenol-enriched fractions or pure compounds with antioxidant, pro-apoptotic or antiproliferative properties to be optimized as anticancer adjuvants.

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Selected Publications

- S. DI MICCO, C. DAQUINO, C. SPATAFORA, F. MAZUÉ, D. DELMAS, N. LATRUFFE, TRINGALI C., R. RICCIO, G. BIFULCO (2010). Structural basis for the potential antitumour activity of DNA-interacting Benzo[*k*]xanthene lignans. *ORGANIC & BIOMOLECULAR CHEMISTRY*, in press
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