

Natural and Synthetic Furanocoumarins as Treatment for Vitiligo and Psoriasis

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Abstract: Phototherapy has been used for centuries to treat various skin disorders. Numerous inflammatory skin diseases, such as atopic dermatitis and pigment disorders like vitiligo and psoriasis, benefit from ultraviolet light treatment.

Psoralen-containing plants have been used for centuries in popular medicine to treat vitiligo, a skin disease characterized by lack of pigmentation. Further advancement in treatments using different psoralen molecules should strive to decrease the possibility of long-term side effects such as cutaneous malignancies. One of the directions for continued refinement of photochemotherapy in the future, as well as one of the new paradigms associated with photochemotherapy itself, is development of other psoralen molecules that do not form bifunctional adducts, which provide a basis for the DNA cross-linking. One such class of furanocoumarins is the methylangelicins (angular furanocoumarins) which only forms monofunctional adducts. There is clearly a theoretical basis that monofunctional adducts would less likely promote cutaneous malignancies as compared to bifunctional adducts.

In this review we wish to present recent pharmacological approaches of furanocoumarins, particularly angular furanocoumarins, and a detailed investigation on the photocytotoxicity exerted by these compounds. Furthermore the edible vegetables and fruits which contain these compounds are showed.

Key Words: Psoriasis, vitiligo, phototherapy, natural and synthetic furanocoumarins.

SKIN DISORDERS

Introduction

The skin is the largest organ of the body. Is an ever-changing organ that contains many specialized cells and structures. The skin functions as a protective barrier that interfaces with a sometimes hostile environment. In addition to serving as a protective shield against heat, light, injury, and infections, the skin also: regulates body temperature, stores water, fat, and vitamin D, can sense painful and pleasant stimulation. The skin is one of the most vulnerable organs of the body. Though seldom life threatening, skin disorders can be uncomfortable and may cause chronic disabilities. In addition, because the skin is so visible, skin disorders can lead to psychological stress [1].

Vitiligo

Vitiligo is an 'idiopathic' acquired depigmenting disorder characterized by the loss of functional melanocytes from the epidermis. Considered the most common pigmentary disorder, vitiligo involves complex interaction of environmental and genetic factors that ultimately contribute to melanocyte destruction, resulting in the characteristic depigmented lesions. In the past few years, studies of the genetic epidemiology of vitiligo have led to the recognition that generalized vitiligo is part of a broader autoimmune disease.

Vitiligo is often present before the age of 20 years and affects approximately 2% of the population. Although the exact etiology is unknown, vitiligo certainly has a genetic component in many patients. Vitiligo has been associated with several autoimmune endocrinopathies, including Grave's disease, Hashimoto's thyroiditis and Addison's disease.

Several types of vitiligo are distinguished according to the distribution of the achromic lesions. Clinically there are five major types of vitiligo: focal, segmental, generalized, acrofacial and universalis. Focal vitiligo is characterized by depigmented patch (or a few patches) in a focal and nondermatomal distribution. Features of the segmental type include depigmented macules in a quasi dermatomal and unilateral distribution. This type of vitiligo has earlier age of onset and usually stabilizes within a year or two of its onset; it's usually not associated with other autoimmune diseases or a familiar pattern of inheritance. The most common form of vitiligo has a generalized distribution. This variety of vitiligo is symmetrical and usually involves the extensor surfaces of the hands, wrists, legs and axillae. In the generalized type, lesions often appear on the face as well and usually in a symmetrical pattern. Acrofacial vitiligo involves the distal digits and the perioral region of the face. The fifth type of vitiligo is universal vitiligo (vitiligo universalis). In this type, the depigmentation is so widespread that only a few areas of normal skin remains. The course of the disease is unpredictable but is often progressive with phases of stabilized depigmentation, whereas segmental vitiligo is relatively stable apart from its explosive onset [2].

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Recent progress in defining the genetic underpinnings of vitiligo has hinged on clearly defining the disorder, thus permitting investigators to test specific hypotheses *via* carefully designed studies. Accordingly, most studies have focused on generalized vitiligo. Generalized vitiligo is defined as an acquired pigmentary disorder characterized by depigmentation due to melanocyte loss in the regions of involved skin, in a pattern that is generally bilateral across the midline, though not necessarily symmetric [3]. This definition includes vitiligo vulgaris (the classic pattern of generalized vitiligo) [4], acrofacial vitiligo (which often progresses to more extensive skin involvement) and vitiligo universalis (complete or almost complete depigmentation). All of these forms are associated with other autoimmune diseases. However, this definition excludes segmental vitiligo and other localized forms of vitiligo, whose true pathogenetic relationship to generalized vitiligo remains unknown.

Melanocyte loss in generalized vitiligo is now widely believed to occur on an autoimmune basis [4-7], although the triggers and specific nature of the autoimmune response remain unknown.

Recently, a new hypothesis called "melanocytorrhagy" advocating trans-epidermal elimination of melanocytes because of a genetically defective adhesion has also been proposed. The autoimmune theory has the most advocates, but the other hypotheses have their own advocates as well. Each theory has its own supporting evidence and from the available data, it is most likely that the loss of follicular and epidermal melanocytes in vitiligo may be the result of several pathogenetic mechanisms acting in unison. As a part of melanocytorrhagic theory, it is suggested that reactive oxygen species (impaired redox status theory) or catecholamines (neural theory) can induce the loss of dendricity of melanocytes, which not only could affect melanosome transfer but also could exaggerate traumatic transepidermal loss of genetically adhesion-deficient melanocytes [8]. Direct actions include interacting with cellular sulfhydryl groups, enzyme inhibition, impairing mitochondrial calcium uptake and forming some cytotoxic products, including free radicals. The indirect effects include activating α -receptors of the arterioles, causing a severe vasoconstriction and thereby producing toxic oxygen radicals caused by hypoxia [9].

It is also demonstrated that hypoxia contributes to the initiation of adaptive immune responses by dendritic cells, favoring the development of Th1 immunity [10]. Moreover, there are several ways by which toxic oxygen radicals, besides having a direct melanocytotoxicity, can induce an autoimmune attack against melanocytes. Some researchers believe that the loss of melanocytes in vitiligo is the result of apoptosis [11]. Oxidative stress, which can induce apoptosis by releasing caspase activating cytochrome C from mitochondria [12], may induce or contribute to the apoptosis of melanocytes in vitiligo lesions. The apparently simple problem of vitiligo is indeed one of the most challenging ones in dermatology and medicine. A good angle of attack to the problem is obviously lacking. When looking at vitiligo skin, the disease seems clinically at least simpler than other common chronic skin disorders, such as atopic dermatitis or psoriasis. There is only a visible loss of pigmentation, and oppo-

site to these other chronic skin conditions, inflammation is lacking.

Psoriasis

Psoriasis is a chronic, inflammatory disease most commonly manifested by well-demarcated, erythematous, silvery-scaled plaques on the elbows, knees, scalp and trunk [13]. Psoriasis is a chronic disease characterized by periods of remission and relapse. Patients with moderate to severe psoriasis, which predominantly presents at a young age, require lifelong treatment to control their disease. Historically, the long-term management of psoriasis has been complicated by a variety of treatment-related factors, including inconsistent efficacy over time, the risk of significant cumulative toxicities, and patient dissatisfaction and noncompliance [14]. To maintain control of psoriasis over the course of the disease despite these limitations, dermatologists typically prescribe numerous therapies and use a variety of management techniques with the aim of reducing individual drug toxicities and avoiding extended treatment-free periods and inevitable return of disease. Prompt transition may allow better continuity of disease suppression, thus improving the patients' quality of life and minimizing the potential for relapse and rebound in patients who must discontinue a particular therapy; however, such an approach has not been evaluated prospectively in a systematic manner for either efficacy or safety.

Psoriasis is mediated by activated T cells [15, 16] and activated dendritic cells found in psoriatic plaques. These cells release inflammatory cytokines, including both tumor necrosis factor- α (TNF) and interferon- γ (IFN- γ). TNF and IFN- γ are over-expressed in plaques and are pro-inflammatory [17]. TNF increases inflammatory cytokines (IL-1, IL-6, NF κ B activation products). It increases blood vessel synthesis (*via* vascular endothelial growth factor), vasodilatation (*via* inducible nitric oxide synthase) and keratinocyte proliferation [15, 18, 19].

New targeted biological therapies which inhibit TNF- α (e.g., etanercept, infliximab) have been successful in the treatment of psoriatic [19, 20]. Increased expression of IFN- γ in circulating T cells of psoriatic patients compared with normal volunteers has been demonstrated with evidence of IFN- γ 's ability to induce psoriatic plaques [15]. Evidence for both genetic and environmental influences has been reported [21-24]. Genetic susceptibility has been linked to class I and class II major histocompatibility complexes on chromosome 6. Two disease subtypes have been proposed, differing in both the age of onset and the human leukocyte antigen (HLA) represented [23].

Psoriasis frequently appears at sites of trauma. A wound induces a reaction characterized by the proliferation of keratinocytes, fibroblasts, vascular elements, nerves, and an accumulation of inflammatory cells. Recent reports suggest that necrosis growth factor (NGF), produced by the keratinocytes, plays a role in wound healing [25, 26]. NGF promotes axonal regeneration and re-innervation of terminal cutaneous nerves. In nonpsoriatics, healing stops after a finite time depending on the nature of the wound. In psoriatics, a wound frequently results in papulosquamous lesions.

Histologically, a psoriatic lesion is characterized by hyperkeratosis, parakeratosis, acanthosis, angiogenesis, neutrophilic microabscesses and lymphomononuclear cell infiltrates. In addition, in the last decade, another characteristic histological feature of psoriasis has been reported, i.e. the hyperproliferation of cutaneous nerves [27, 28].

Plaque is the most common form of psoriasis, ranging from 34% to 84% in frequency in several large studies [29, 30]. This variation may be the result of the exclusion of psoriatic rash, scalp, facial and anogenital involvement from the "plaque" category in some studies. Plaque psoriasis consists of well-demarcated, erythematous lesions with silvery-white scales most commonly found on the extensor surfaces, knees, buttocks, elbows and scalp. The scalp is the most frequently involved region and is the site of onset in 40-60% of patients younger than 20 years of age.

The skin responds to numerous psychological stimuli. It is accepted by most dermatologists that psoriasis is exacerbated by stressful life events. There is a complex relationship between the mind and the skin, ranging from the stigmatization of disfiguring skin lesions to the cutaneous effects of functional and organic diseases. The time between periods of increased stress and exacerbation of psoriasis is usually less than 1 month, and in two thirds of the patients, it occurred within 2 weeks. Ingram believed that emotional stress was the most potent precipitating factor in psoriasis. Patients with psoriasis are more responsive to stress, at least in terms of the sympathoadrenomedullary system. Psychoneuroimmunological mechanisms may provide an explanation for the correlation between stress and psoriasis.

PHOTOTHERAPY

Treatment of skin diseases using artificial sources of ultraviolet (UV) radiation has been practised in hospitals since the early part of the 20th century following the pioneering work of the Danish physician Niels Finsen [31] who was awarded the Nobel Prize in 1903. Treatment was by exposure to UV lamps where the therapeutic waveband was largely in the UVB (280–315 nm) region and often used in combination with agents applied topically such as coal tar or dithranol [32]. The therapeutic response and duration of remission for this treatment still comprise one of the standards against which new therapies are measured; however, the long-term hospital admissions of 2 to 3 weeks and the many hours of special wrappings on the skin required by the treatment make this procedure very time-consuming, demanding and expensive to deliver appropriately. Likewise, the Ingram method, which uses short-term contact with anthralin in combination with ultraviolet B therapy, has also proven to be a very effective treatment for psoriasis, but again requires specialized nursing facilities and day-long care or hospitalization. A more recent change in the paradigm for phototherapy use emerged in the second half of the 20th century from the recognition and implementation of photochemotherapy for treatment of skin diseases. The combination of a photon-absorbing chemical delivered in a systemic manner, followed by delivery of ultraviolet light to the skin, was observed to promote clearing of psoriasis in a high percentage of patients.

It is through just such discoveries by keen observation that fundamental change and advancement in a discipline can occur. The paradigm of using ultraviolet light in combination with an endogenous or exogenous chemical to promote a photochemical reaction changed the entire approach for the concept of photochemotherapy and photodynamic therapy. In North America, the leaders in the development of photochemotherapy were Drs. Fitzpatrick and Parish. Their observations of the effects of photochemotherapy on vitiligo and subsequently the notable improvement in psoriasis on the skin of a patient undergoing photochemotherapy led to the further development and application of PUVA treatments. PUVA is one of the best examples of advancement of dermatologic therapy in the last quarter of the 20th century. The most widely used therapeutic modalities include: broadband UVB (290–320 nm), narrow-band UVB (311 ± 2 nm), 308 nm UVB excimer laser, UVA, psoralen plus UVA (PUVA), combined UVA–UVB, high-dose UVA1 and high dose visible light.

UV irradiation has been shown to influence the synthesis and release of inflammatory mediators from different cell types [33]. The effect of *in vitro* UVA irradiation of basophils is characterized by a biphasic dose dependent action on histamine release: low doses are followed by a significant inhibitory effect, in contrast high doses are followed by histamine liberation [34]. Guhl *et al.* have recently demonstrated that UV light also has a dual effect on mediators release from dermal mast cells [35]. They found that UVB, UVA1 and PUVA irradiation resulted in a slight release of histamine from resting mast cells and in a dose-dependent inhibition of the release of histamine and tryptase from activated cells. All three types of irradiation also resulted in a strong inhibition of the *de novo* synthesis of pro-inflammatory mediators (IL-6 and IL-8), irrespective of the activation status of mast cells. Moreover, UV irradiation fully inhibited the stimulatory capacity of anti-IgE on TNF- α production. UV light also has an important effect on cytokine release from T cells. UVB radiation suppresses the production of pro-inflammatory cytokines IFN- γ , IL-12 and IL-2 and induces the secretion of immunomodulatory cytokines such as IL-10, which has been also implicated in tolerance induction [36]. Schwartz *et al.* have demonstrated that cutaneous application of haptens to UV-exposed skin induced hapten-specific tolerance which is mediated through IL-10 producing T regulatory cells (Tr1) [37]. Recently, they have also showed that UV-induced Tr1 can suppress the effector phase of contact hypersensitivity [38].

The therapeutic effect of UV light is mostly attributed to its immunosuppressive and immunomodulant action [39]. One of the most important mechanisms that explain the immunosuppressive effect of UV light is apoptosis induction. Krueger *et al.* observed that UVB treatment of psoriatic skin lesions resulted in a consistent and profound depletion of T lymphocytes from the epidermis and to a much lesser extent from the dermis. Ozawa *et al.* showed that the primary mechanism of depletion of skin infiltrating T cells was UVB-induced apoptosis [40]. *In vitro* experiments also demonstrated that UVB light has a profound effect on apoptosis induction of different cells types (lymphocytes, keratinocytes, eosinophils). Novak *et al.* performed a systematic

evaluation of the apoptosis inducing capacity of different UVB light sources [41]. They found that apoptosis inducing capacity in T cells decreased from 290 to 311 nm wavelengths. They also demonstrated that apoptosis induction of a monochromatic, high irradiance light source, the XeCl laser (308 nm), is much higher compared to other light sources, suggesting that irradiance also influences the effect of UVB radiation. UVA irradiation has been also demonstrated to induce T cells apoptosis both *in vivo* and *in vitro*. In particular, UVA1 (340–400 nm) proved to be effective in inducing apoptosis of T helper cells [42].

Induction of programmed cell death or apoptosis has been extensively studied in the last few decades. One of the main mechanisms by which UV light induces apoptosis is UV induced DNA damage [39]. UV irradiation leads to the formation of pre-mutagenic lesions, called photoproducts (cylobutane pyrimidine dimer, 6–4 photoproduct formation, Dewar isomers). These photoproducts are the major triggers of UV induced apoptosis. Matsunaga *et al.* determined the action spectrum for the induction of photoproducts and found that the most efficient wavelength was 260 nm, corresponding to the absorption spectrum of DNA [43]. Another mechanism for apoptosis induction is the capacity of UVB to directly activate death receptors such as CD95 (Fas) by inducing receptor clustering without the need of its ligand [44]. Clustering of the receptors results in activation of the death domain and initiates apoptosis. Induction of reactive oxygen species after UVB and/or UVA irradiation might be also responsible for apoptosis induction [45]. All these pathways may contribute in an additive manner to UV induced apoptosis. UV irradiation has a profound effect on antigen presentation. It has been shown that UVB and UVA irradiation resulted in depletion of epidermal Langerhans cells and reduced antigen presenting capacity of epidermal antigenpresenting cells [46]. Moreover, UVA has been recently shown to impair the phenotypic and functional maturation of migrating dermal dendritic cells into potent antigenpresenting cells and to induce their apoptosis [47]. The mixed epidermal lymphocyte reaction (MECLR) and the mixed lymphocyte reaction (MLR) have been commonly used to study the immunosuppressive effect of UV radiation. Both MLR and MECLR responses decrease after UVB exposure [48].

Psoriasis, a disease characterized by red, scaly plaques and affecting about 2% of the population, is the skin disease most frequently treated by PUVA. However there are many other disorders that show partial or complete response to PUVA and these include vitiligo, eczema, lichen planus, graft-versus-host disease, cutaneous T-cell lymphoma (mycosis fungoides) and photosensitive disorders such as polymorphic light eruption, actinic prurigo and chronic actinic dermatitis [49].

PHOTOCHEMOTHERAPY

Psoralen photochemotherapy is the combined treatment of skin disorders with a photosensitizing drug (Psoralen) and UltraViolet A radiation (315–400 nm). Psoralens are naturally occurring plant compounds and their therapeutic potential for the treatment of the socially disfiguring disease vitiligo has been recognized for many thousands of years [50]. Photochemotherapy using psoralen compounds such as 8-

methoxypsoralen (8-MOP) or, to a lesser extent, 5-methoxypsoralen (5-MOP) was introduced in the 1970s [51].

When PUVA therapy was introduced, the dose of 8-MOP was calculated on body weight and given in a standard dose of 0.6 mg kg^{-1} [49]. As nuclear medicine physicists know, body surface area shows a higher correlation with plasma volume than does body weight and it was suggested that a more appropriate dosing of 8-MOP might be achieved using a system based on surface area, and provided evidence [52] that this method of dosing at 25 mg m^{-2} improved the therapeutic effect of PUVA in psoriasis.

Following oral administration of 8-MOP, absorption and resulting plasma concentrations show considerable variation between subjects, but UVA exposure is given usually 2 h after ingestion at the average time of peak plasma concentration [53]. PUVA may also be given using topical psoralen, either painted onto the skin surface or, more frequently, using a bath delivery system in which the patient soaks for 15 min in a weak psoralen solution e.g. 3.75 mg l^{-1} of 8-MOP, followed immediately by UVA exposure. Significant concentrations of psoralen in plasma are not achieved with topical psoralen. Psoralen molecules, when activated by UVA radiation form cross-links between adjacent strands of DNA, thus interfering with DNA and cellular replication. Although it has been assumed that this is the mechanism of action of PUVA in disorders associated with increased cell division (such as psoriasis), PUVA also has other important actions on the skin, including induction of pigmentation and epidermal hyperplasia, suppression of certain components of the immune system and release of reactive oxygen and free radicals which damage cell membranes and cytoplasmic structures.

Further advancement in treatments using different psoralen molecules should strive to decrease the possibility of long-term side effects. The most important long-term side effect demonstrated with the use of PUVA therapy has been the development of cutaneous malignancies, which occurred after cumulative high dose therapy extended over time. For example, patients having had more than 200 PUVA treatments are at increased risk for developing squamous cell carcinomas.

This is especially an important consideration with regard to the areas of male genitalia, which is more prone to having squamous cell carcinoma in relation to PUVA therapy [54]. This effect from PUVA seems to be due to a cumulative dose. There is an induction period for the development of squamous cell carcinomas, which appears to be 10–15 years.

Evidence reported from the North American cohort of PUVA patients followed over the past 25 years demonstrated an increased frequency of development of melanoma in certain groups [55]. It is important that patients having a Type I Fitzpatrick or Type II Fitzpatrick do not receive excessive exposures to photochemotherapy. In addition to patients with Type I or Type II skin being at increased risk, patients who have received greater than 200 PUVA treatment are also likely to have an increased chance of developing melanoma. Finally, patients who have received a high number of treatments and experienced a 15- to 20-year latency period

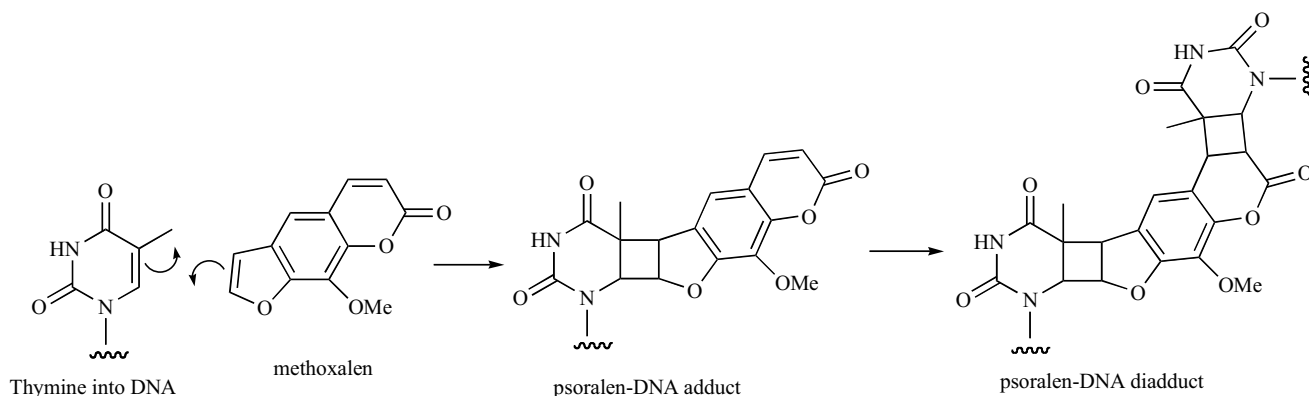


Fig. (1). Intercalation and DNA-photobinding.

should be followed because of a possible increased incidence of melanoma.

A component of the theoretic basis for such incidence of squamous cell carcinoma in the high-dose group of PUVA patients are the photobiologic effects of PUVA on DNA. Psoralen molecules can enter the nucleus of cells and intercalate between DNA base pairs. With absorption of a photon of light, a covalent bond is formed with adjacent pyrimidine bases, producing a cyclobutane ring. If a second photon of light is absorbed by the psoralen molecule when it already has had formation of a cyclobutane ring, a DNA cross-link is formed across the strands of DNA base pairs (Fig. 1). Theoretically, this is the prime basis for repetitive errors in DNA and plays a role in formation of squamous cell carcinoma. The influence of the direct DNA damage in the pathogenesis of malignancy may be either a direct effect on the keratinocytes or indirect through abnormalities in the altered immunologic surveillance of the skin.

FURANOCOUMARINS

Furanocoumarins are a group of natural and synthetic compounds used for the photochemotherapeutic treatment of some skin diseases, some lymphomas and autoimmune disorders [56, 57]. Furocoumarins have pharmacological properties which are active even without irradiation; they show some activity against psychological depression and also seem to be useful drugs for the treatment of multiple sclerosis, because of their ability to block potassium channels [58]. The antiproliferative activity of these compounds is connected with their capacity to photoinduce selective lesions to DNA. Although this mechanism is very effective in treating some diseases such as psoriasis and mycosis fungoides, some side-effects have been observed, e.g. risk of cancer, skin phototoxicity and others [59].

Retrospective studies of PUVA patients show significantly increased incidence of cutaneous melanoma. For the past 50 years cutaneous melanoma is increased at similar rates as the increased availability and consumption of *Citrus* products. Recently in a large study of nurses, only orange juice drinking, indicative of dietary preference for *Citrus*, was positively associated with significantly increased risk of developing cutaneous melanoma. Sayre and Dowdy [60] hypothesized that the increases in cutaneous melanoma inci-

dence may be in part related to concomitant increases in dietary photocarcinogenic furocoumarins.

One of the directions for continued refinement of photochemotherapy in the future, as well as one of the new paradigms associated with photochemotherapy itself, is development of other psoralen molecules that do not form bifunctional adducts, which provide a basis for the DNA cross-linking.

NATURAL ANGULAR FURANOCOUMARINS

Furanocoumarins are biologically active natural compounds found mainly in plants belonging to the Umbelliferae and Rutaceae such as celery (*Apium graveolens*), carrots (*Daucus carota*) and parsnips (*Pastinaca sativa*), but also in plants of Rutaceae, Apiaceae, Asteraceae, Fabaceae, Oleaceae, Moraceae and Thymeleaceae families. The natural occurrence of biologically active furanocoumarins in common vegetables is an area of increasing interest with respect to human health. In fact, among the several biological properties of coumarins there are dermal photosensitising, estrogenic, antimicrobial, vasodilator, molluscicidal, antihelminthic, sedative and hypnotic, analgesic and hypothermic activities [61] but they have been associated mainly with anticoagulant activity [62]. One of the most known natural angular furanocoumarins was angelicin (**A1**) (Fig. 2) isolated from different plant species such as *Angelica* species, *Citrus limonia*, *Psoralea corylifolia*, *Elaeagnus bockii* *Diplotaenia damavandica* [63-70].

The search of potential therapeutic agents in hematological diseases, including β -thalassemia and sickle cell anemia, focuses on the pharmacologically-mediated regulation of the expression of human γ -globin genes [71, 72]. The angular type furanocoumarin **A1** was tested to evaluate whether it is able to increase the expression of γ -globin genes in human erythroid cells [73]. To verify the activity of **A1**, two experimental cell systems, the human leukemic K562 cell line and the two-phase liquid culture of human erythroid progenitors isolated from normal donors, were employed. **A1**, compared with cytosine arabinoside, mithramycin and cisplatin, is a powerful inducer of erythroid differentiation and γ -globin mRNA accumulation of human leukemia K562 cells. In addition, when normal human erythroid precursors were cultured in the presence of **A1** increases of γ -globin mRNA accumulation and fetal hemoglobin (HbF) production, even

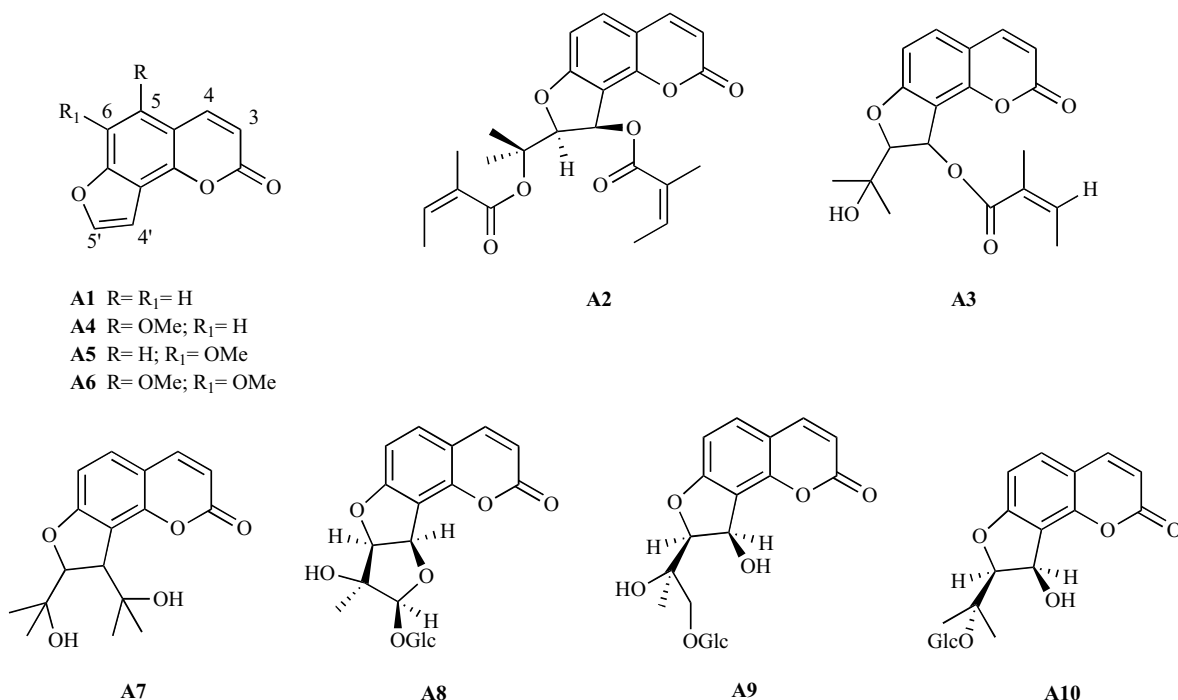


Fig. (2). Natural angular furanocoumarins.

higher than those obtained using hydroxyurea, were detected. These results could have practical relevance as pharmacologically-mediated regulation of the expression of human γ -globin genes, leading to HbF induction. This is considered a potential therapeutic approach in hematological disorders, including β -thalassemia and sickle cell anemia.

The bioactivity-guided fractionation of the active dichloromethane extract of *Psoralea glandulosa* yielded the isolation of angelicin (**A1**) that was tested for its anti-inflammatory and antipyretic activities [74]. At the dose of 20 mg/kg, **A1** showed 39.0% of anti-inflammatory effect and at the dose of 17 mg/kg 68% of antipyretic effect. Angelicin (**A1**), and three linear furanocoumarins libanorin, psoralen and auraptene were isolated from the methanol extract of *Diplotaenia damavandica* and tested for their cytotoxicity against the human KB cell line and for their antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Cladosporium cucumerinum* [66]. The main antifungal component of *D. damavandica* is the angular furanocoumarin angelicin (**A1**).

A mixture (1:1) of angelicin (**A1**) and psoralen, isolated from the seeds of *Psoralea corylifolia* exhibited significant activity against Gram (+) *Staphylococcus aureus* [69].

Potent anti-tumor promoter activity has been found in the non-polar extracts of the root of "Ashita-Ba", *A. keiskei* (Umbelliferae), which is eaten as a vegetable in Japan [75]. From this active fraction, two angular furanocoumarins, archangelicin (**A2**) and 8(*S*),9(*R*)-9-angeloyloxy-8,9-dihydro-oroselol (**A3**) together with other compounds were isolated. Among tested compounds the two angular type furanocoumarins suppressed 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-stimulated 32Pi-incorporation into phospholipids of cultured cells.

Furanocoumarins are typical phototoxic compounds leading to photodermatitis in combination with UV light exposure [76]. Furthermore, furocoumarins are cytotoxic and mutagenic in mammalian cells in culture [76]. On the molecular level furocoumarins bind to cellular constituents such as proteins, lipids, etc., can damage lysosomes, lead to the formation of reactive oxygen species and can contribute to the formation of novel antigens by covalent modification of proteins [77]. Furthermore, furocoumarins are well known for their interference with drug metabolism, in particular with cytochromes P450 (CYP). The monomeric 6',7'-dihydroxybergamottin and related dimers are highly potent inhibitors of CYP3A4 and other CYP enzymes involved in the metabolism of many drugs. Imperatorin and isopimpinellin act as inhibitors of 7-pentoxoresorufin *O*-deethylase (PROD) activity catalyzed by CYP2B enzymes, while bergamottin and coriandrin inhibit 7-ethoxyresorufin *O*-deethylase (EROD) activity catalyzed by CYP1A enzymes [78]. Recently, Baumgart *et al.* [79] investigated the relative potencies of three linear furocoumarins bergamottin, isopimpinellin and 8-methoxypsoralen (8-MOP) and the angular furanocoumarin angelicin (**A1**) as inhibitors and inducers of CYP1A1 and/or EROD activity in rat hepatocytes in primary culture. In particular, experiments were carried out in the presence or absence of light to differentiate between effects of furocoumarins after external activation by light and light-independent effects on drug metabolism. Effects on CYP1A gene expression were analyzed on the levels of mRNA, XRE-driven reporter gene expression and enzyme protein. Angelicin (**A1**) led to a complete inhibition of EROD activity, independent of the mode of addition of the inhibitor, either over 48 h to intact hepatocytes together with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or for 10 min prior to addition of NADPH to microsomes prepared from TCDD-treated cells. In intact

hepatocytes, 8-MOP was more potent in the dark than in the presence of light, while the opposite was found for bergamottin. For angelicin and isopimpinellin, no significant differences were found. In microsomes, all furanocoumarins including **A1** were potent inhibitors when light was present, the difference in potency between light and dark being about six-fold for bergamottin, 12-fold for 8-MOP, 18-fold for isopimpinellin and 38-fold for angelicin (**A1**). Methoxyporalen and angelicin (**A1**) led to a significant induction of CYP1A1 mRNA in hepatocytes, while all furanocoumarins except bergamottin increased xenobiotic-responsive element-driven reporter gene expression in transfected H4IIE rat hepatoma cells when light was excluded. Furthermore, all furocoumarins tested induced the expression of endogenous, immunoreactive CYP1A1 protein, primarily in the dark. These results demonstrate that tested furocoumarins can interfere with AhR-regulated CYP1A1 expression and activity in at least three major ways, i.e., (i) acting as highly potent inhibitors of the catalytic activity of CYP1A1 both in the presence and absence of light, (ii) inducing CYP1A1 gene expression in the absence of light *via* activation of the AhR, and (iii) inducing CYP1A1 gene expression without activation of the AhR.

Inducible nitric oxide synthase (iNOS)-dependent production of nitric oxide (NO) plays an important role in inflammation. The effects of various naturally occurring furanocoumarins, isolated from Umbelliferae or Rutaceae medicinal herbs, on NO production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophage cells were evaluated *in vitro* [80]. Angelicin (**A1**), which is the fundamental skeleton lacking substitutions on the benzene ring, showed a moderate inhibitory effect (53.6%) on LPS-induced NO generation in RAW 264.7 cells. The activity of isobergaptin (**A4**), with a methoxy group at the C5 position, exhibited a considerably lower inhibitory effect (8.8%), whereas sphondin (**A5**), with a methoxy group at the C6 position, showed a strong inhibitory effect. The activity of pimpinellin (**A6**), having two methoxy groups at the C5 and C6 positions, was slightly higher than that of **A1**. On the basis of this evidence, a methoxy group at the C6 position of the angular furanocoumarin skeleton seems to be very important for activity and the addition of a methoxy group at C5 decreases activity. Sphondin (**A5**) most strongly inhibited NO production and iNOS expression in LPS-induced RAW 264.7 macrophages as compared to the other furanocoumarins tested. This notion is based on the following lines of evidence: (1) the least nitrite accumulated in cell supernatants when **A5** was added simultaneously with LPS; a delayed addition resulted in a decreased effect; (2) **A5** did not inhibit NO accumulation after LPS stimulation of RAW 264.7 cells and did not show a direct effect on enzymatic activity of iNOS; (3) western blot analyses demonstrated markedly reduced levels of iNOS protein in LPS-activated cells treated with **A5** as compared to untreated cells.

P-glycoprotein (Pgp) is an inducible protein transporter belonging to the ATP-binding cassette (ABC) protein family that mediates the energy-dependent efflux of many drugs, including chemotherapeutics, out of cells. In cancer cells, the overexpression of Pgp encoded by the MDR1 gene contributes to multidrug resistance (MDR) and is already consid-

ered as one of the major obstacles to successful cancer chemotherapy. The observation that numerous plant-derived dietary compounds modulate Pgp transport has led to interest in the possible use of natural compounds, or related chemicals, in combination with chemotherapy. Using MDCK-MDR1 cells as a model of cells expressing the human MDR1 phenotype, cnidiadin (**A7**), isolated from *Tordylium apulum*, was tested for its capacity to induce the accumulation of two Pgp substrates (the lipophilic cation R-123 and the radiolabeled anticancer agent [3 H]-VBL) and compete with a CsA analogue for binding to Pgp. CsA and verapamil were used as positive controls. **A7** was capable of significantly inhibiting the extrusion of the lipophilic cation R-123 and the radiolabeled anticancer agent [3 H]-VBL out of MDCK-MDR1 cells [81]. At high concentrations (100 μ M), **A7** induced the accumulation of R-123 more efficiently than the two positive controls and was slightly more potent than CsA in its ability to induce the accumulation of [3 H]-VBL. These results indicate that **A7** can inhibit Pgp transport and may act as a reversing agent. Interestingly, in the dose range 0-100 μ M, **A7** substantially increased [3 H]-VBL uptake in a dose-dependent manner. The maximal accumulation (7.2 times the control level) was close to the accumulation (7.0 times) promoted by 10 μ M CsA. At low concentrations, **A7** (IC₅₀ 26.4 μ M) is however a less potent reversal agent than CsA (IC₅₀ 3.2 μ M). Nevertheless, 10 μ M **A7** effectively induces the accumulation of [3 H]-VBL in this cell-line. The demonstration that this effect is due to a competition for the binding to Pgp was shown by a correlation between the dose-response curves of photolabeling inhibition and of [3 H]-VBL accumulation. Consistent with a specific inhibition of Pgp efflux, [3 H]-VBL uptake was not increased by an inhibitor of MRP in the absence of **A7** and was only slightly increased when this inhibitor was used in combination with **A7**. These results support the hypothesis that in MDCK-MDR1 cells the accumulation of [3 H]-VBL following treatment with cnidiadin (**A7**) is almost completely specific to a competitive inhibition of Pgp transport activity. This effect is in a large part due to the two hydroxyisopropyl chains linked on the dihydroangelicin nucleus. To further evaluate the potency of **A7** as a reversal agent, its capacity to sensitize MDCKMDR1 and KB/VCR cells to vinca alkaloids was tested. Cnidiadin (**A7**) is a cytotoxic agent and this is in agreement with previous findings in nonsmall-cell lung carcinoma (NSCLC) N6 cells, showing that **A7** is a cell-cycle inhibitor blocking cells in the G1 phase [82]. MDCKMDR1 cells appear much more susceptible to cnidiadin (**A7**) than N6 cells. Indeed, a single treatment with 10 μ M **A7** decreased the survival of this cell line by 50%. Used together with 0.6 μ M vincristine (VBL), 10 μ M **A7** increased the cell toxicity of VBL by 163%. This finding indicates sensitization of this resistant cell line to VBL, but does not permit the evaluation of cell toxicity. To evaluate a possible benefit of cnidiadin (**A7**) in the treatment of human resistant tumors overexpressing Pgp, its capacity to enhance the cell toxicity of vincristine (VCR) in KB/VCR cells was also evaluated. This mutant human epidermoid carcinoma cell line that expresses the MDR1 gene exhibits high resistance (105-fold) to VCR and mild susceptibility to **A7** (IC₅₀ 43.5 μ M). No cell toxicity was detected when **A7** was used at 10 μ M as a single agent. However, at this non-toxic concentration, **A7** decreased by 24.6% the IC₅₀ value of

VCR in KB/VCR cells. The beneficial effect of the co-treatment supports a synergism between VCR and **A7**. The exact mechanisms by which **A7** potentiates VCR, the toxicity of this furanocoumarin in normal cells and its specificity for cancer cells remains to be evaluated. However, the results clearly establish that co-treatment of KB/VCR cells with VCR and **A7** at non-toxic and toxic concentrations sensitized this resistant cell line to VCR. Considering the important role of Pgp in the oral bioavailability of drugs, it is possible that **A7** in food or folk medications may play a complementary role against MDR by reversing barriers to drug availability. Indeed, Pgp is present in the intestine in the brush border of mature enterocytes where it pumps Pgp substrates from the enterocytes back to the intestinal lumen, preventing their absorption into blood. It has been reported previously that ingestion of furanocoumarins from grapefruit juice increases the bioavailability of Pgp substrates by partially inhibiting intestinal Pgp transport activity. Inhibiting Pgp-mediated drug efflux may have beneficial consequences, but may also contribute to drug interactions. *In vivo* studies were realised to evaluate if ingestion of food or folk medicine containing cniadin (**A7**) has positive or deleterious effects. This study demonstrated that **A7** is a cytotoxic compound and a Pgp substrate capable *in vitro* of competitively inhibiting the binding and efflux of drug by Pgp. The tumoricidal activity of this furanocoumarin and its capacity to sensitize resistant cells overexpressing Pgp to vinca alkaloids suggest that diet and traditional preparations containing **A7** may contribute to (1) reverse multidrug resistance encoded by the MDR1 gene, and (2) increase the bioavailability of orally administered chemotherapeutic agents in humans. Due to its cell toxicity and weak reversal activity at low concentrations, clinical interest in cniadin (**A7**) as a reversal drug may, however, be limited. Recent study afforded to the isolation of two novel angular-type furanocoumarin glycosides, peucedanoside A (**A8**) and peucedanoside B (**A9**), along with a known compound apterin (**A10**), from the roots of *Peucedanum praeruptorum* Dunn. (Umbelliferae) [83].

SYNTHETIC ANGULAR FURANOCOUMARINS

Methylangelicins

In order to reduce the risks of PUVA treatment various methylangelicins were prepared. Methylangelicins are monofunctional furanocoumarins which do not photoinduce interstrand cross-linkages in DNA but which photobind with macromolecules only as monoadducts (Fig. 3).

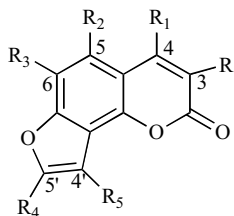
Bordin *et al.* [84] studied 5-methylangelicin (**M1**) in 2 different biological systems, the T2 phage and Ehrlich ascites tumor cells. In comparison with angelicin (**A1**), this compound was several times more active.

A derivative of 4,5'-dimethylangelicin (**M2**) with a long chain linking an amino group to the planar furanocoumarinic moiety, that is 4'-*N,N*-dimethylaminoethoxymethyl-4,5'-dimethylangelicin (**M3**), is able to form effectively the intercalated complex with DNA like the previously prepared 4'-aminomethyl-4,5'-dimethylangelicin (**M4**). However while the compound **M4** shows very poor photobinding to DNA, **M3** shows high photobinding to the macromolecule. Some photophysical data of **M4** and **M3** appear to confirm the

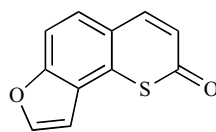
critical role of the position assumed by the chromophore of the two compounds when intercalated in duplex DNA. The compound **M3** displays high photobiological effects, also in terms of antiproliferative activity as shown by its capacity to inhibit DNA and RNA synthesis in Ehrlich cells, the growth of an *E. coli* culture and the infectivity of T2 phage. The compound **M3** on the basis of these properties seems to deserve a clinical evaluation of its potential photochemotherapeutic activity in the treatment of psoriasis [85]. Angelicin (**A1**) and 4,5'-dimethylangelicin (**M2**) were studied for their mutagenic activity in the HGPRT system on V79 chinese hamster cells in culture. These compounds, when activated by near-ultraviolet (NUV) light, were effective in inducing HGPRT mutants. 4,5'-Dimethylangelicin (**M2**) showed greater efficiency than **A1** [86].

Cristofolini *et al.* [87] tested three series of methylangelicins in clearing lesions of psoriasis with topical application and UVA irradiation in 17 patients. The first series, 5-methyl and 4,5-dimethylangelicin, showed less therapeutic activity than 8-MOP [88]. The second series, the 4'-methylangelicins, showed a good antiproliferative activity and a greater therapeutic effect than 8-MOP. However, these compounds showed some skin phototoxicity on guinea-pig and human skin [89, 90]. Another important problem related to the synthetic procedure of the above angelicin derivatives was their purity. The introduction of a methyl group in the 6-position seemed to be a good solution [91], and a third series of compounds were therefore prepared: the 6-methylangelicins. Some of these efficiently photobind to DNA and show a marked therapeutic effect [89, 91]. The photomutagenic activity of two of these compounds (6,4'-DMA (**M5**) and 6,4,4'-TMA (**M6**)) was also investigated and compared with 8-MOP on three biological systems, i.e. *Escherichia coli* WP2 uvrA, Chinese hamster V79 cells and *Aspergillus nidulans* conidia. The most active compound was 6,4,4'-trimethylangelicin (**M6**), which showed a high antiproliferative effect and reduced genotoxicity in comparison with 8-methoxypsoralen (8-MOP). Although the pathogenesis of psoriasis is unknown it is a hyperproliferative disorder of the epidermis and a drug capable of inducing an antiproliferative effect may therefore be effective against psoriasis. The methylangelicins also cause skin pigmentation. The 6-methylangelicins examined by Cristofolini *et al.* [87] showed marked photosensitizing activity. They showed marked inhibition of epidermal DNA synthesis in the mouse, when given both topically and orally and the most active compounds was **M6**. In the photomutagenicity studies, **M5** and **M6** appeared to be less genotoxic than 8-MOP in *E. coli* WP2 uvrA, while in Chinese hamster V79 cells, at the same survival level, they showed similar mutagenicity. However, it should be observed that the same lethal effect was obtained at lower UVA doses than with 8-MOP. These 6-methylangelicins, and in particular **M6** were effective in clearing psoriasis in 17 patients when applied topically and with UVA irradiation. These drugs appeared to be more active than 8-MOP. These compounds did not induce phototoxicity, thus allowing it to be topically applied and avoiding side-effect due to systemic administration.

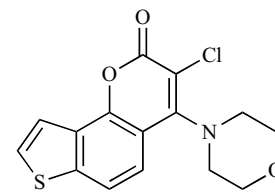
The effect of the introduction of one, two or three methyl groups at the level of 3,4 (**M7**) or 4',5'(**M8**) photoreactive



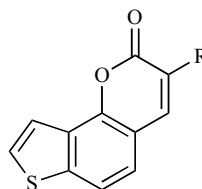
- M1** R = R₁ = R₃ = R₄ = R₅ = H; R₂ = Me
M2 R = R₂ = R₃ = R₅ = H; R₁ = R₄ = Me
M3 R₁ = R₄ = Me; R₅ = N(CH₂NH₂)CH₂OCH₂CH₃
M4 R₁ = R₄ = Me; R₅ = CH₂NH₂
M5 R = R₁ = R₂ = R₄ = H; R₃ = R₅ = Me
M6 R = R₂ = R₄ = H; R₁ = R₃ = R₅ = Me
M7 R = R₁ = Me; R₂ = R₃ = R₄ = R₅ = H
M8 R = R₁ = R₂ = R₃ = H; R₄ = R₅ = Me
M9 R = R₂ = R₃ = H; R₁ = R₄ = R₅ = Me
M10 R = R₃ = R₄ = H; R₁ = R₅ = Me; R₂ = OMe
M11 R = R₅ = Me; R₁ = R₃ = R₄ = H; R₂ = OMe



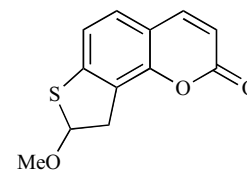
S1



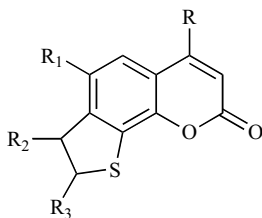
S2



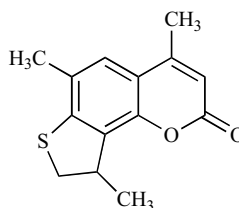
- S3** R = COOCH₃
S4 R = COOC₂H₅
S5 R = COOH
S6 R = H



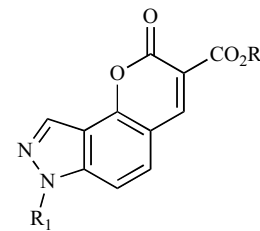
S7



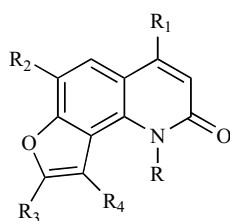
- S8** R = R₂ = R₃ = Me; R₁ = H
S9 R = R₁ = R₂ = R₃ = Me



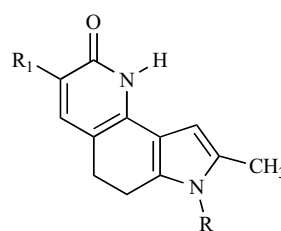
S10



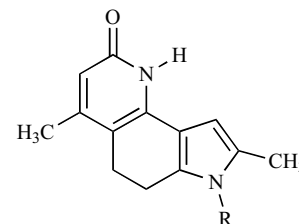
- N1** R = CH₃; R₁ = CH₃
N2 R = C₂H₅; R₁ = CH₃
N3 R = CH₃; R₁ = C₆H₅
N4 R = C₂H₅; R₁ = C₆H₅



- N5** R = R₁ = R₂ = R₃ = CH₃; R₄ = H
N6 R = H; R₁ = R₂ = R₃ = R₄ = CH₃
N7 R = R₄ = H; R₁ = CH₂OH; R₂ = R₃ = CH₃
N8 R = R₂ = R₃ = CH₃; R₁ = CH₂OCH₃; R₄ = H
N9 R = R₂ = R₃ = CH₃; R₁ = CH₂OH; R₄ = H
N10 R = R₂ = R₃ = H; R₁ = R₄ = CH₃
N11 R = R₃ = H; R₁ = R₂ = R₄ = CH₃
N12 R = R₂ = H; R₁ = R₃ = R₄ = CH₃
N13 R = R₄ = H; R₁ = R₂ = R₃ = CH₃
N14 R = H; R₁ = R₂ = R₃ = R₄ = CH₃
N15 R = R₁ = R₃ = CH₃; R₂ = R₄ = H

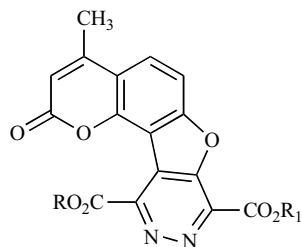


- N16** R = Ph; R₁ = SO₂Ph
N17 R = Ph; R₁ = COPh
N18 R = Ph; R₁ = CN
N19 R = Ph; R₁ = COOEt
N20 R = Me; R₁ = SO₂Ph
N21 R = Me; R₁ = COPh
N22 R = Bn; R₁ = SO₂Ph
N23 R = Bn; R₁ = COPh

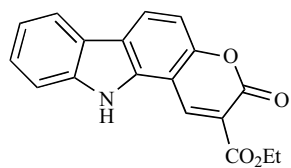


N24

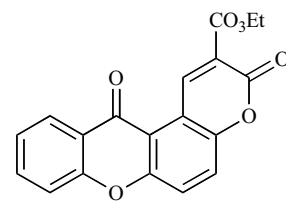
(Fig. (3). Contd....)



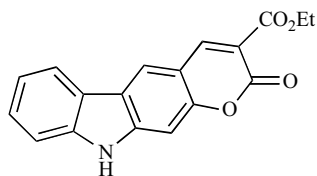
- T1** R = R₁ = Me
T2 R = OMe; R₁ = N(CH₂)₄
T3 R = R₁ = N(CH₂)₄
T4 R = OMe; R₁ = NH(CH₂)₂NMe₂
T5 R = R₁ = NH(CH₂)₂NMe₂
T6 R = NH(CH₂)₂NMe₂; R₁ = N(CH₂)₄



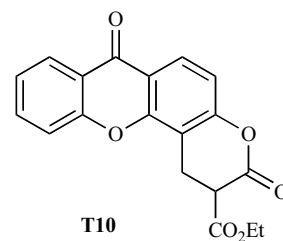
T7



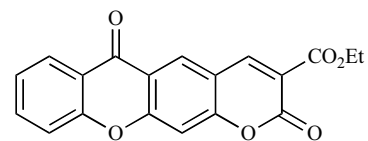
T9



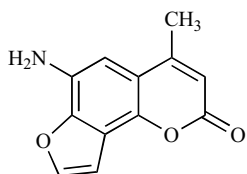
T8



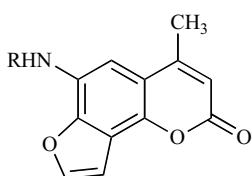
T10



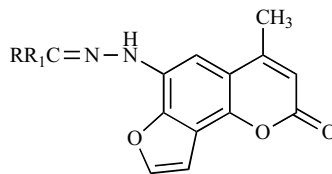
T11



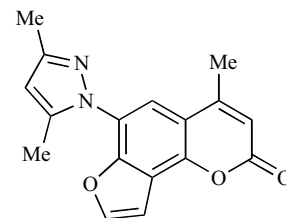
AA1



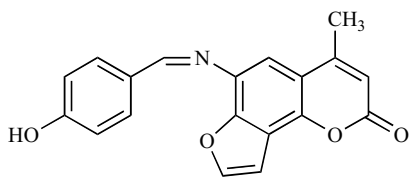
- AA2** R = COMe
AA3 R = COH
AA4 R = Ts



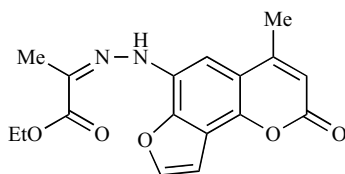
- AA6** R = 4-O₂N-C₆H₄; R₁ = H
AA7 R = 4-HO-C₆H₄; R₁ = H



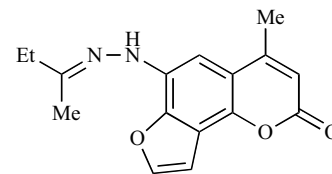
AA10



A5

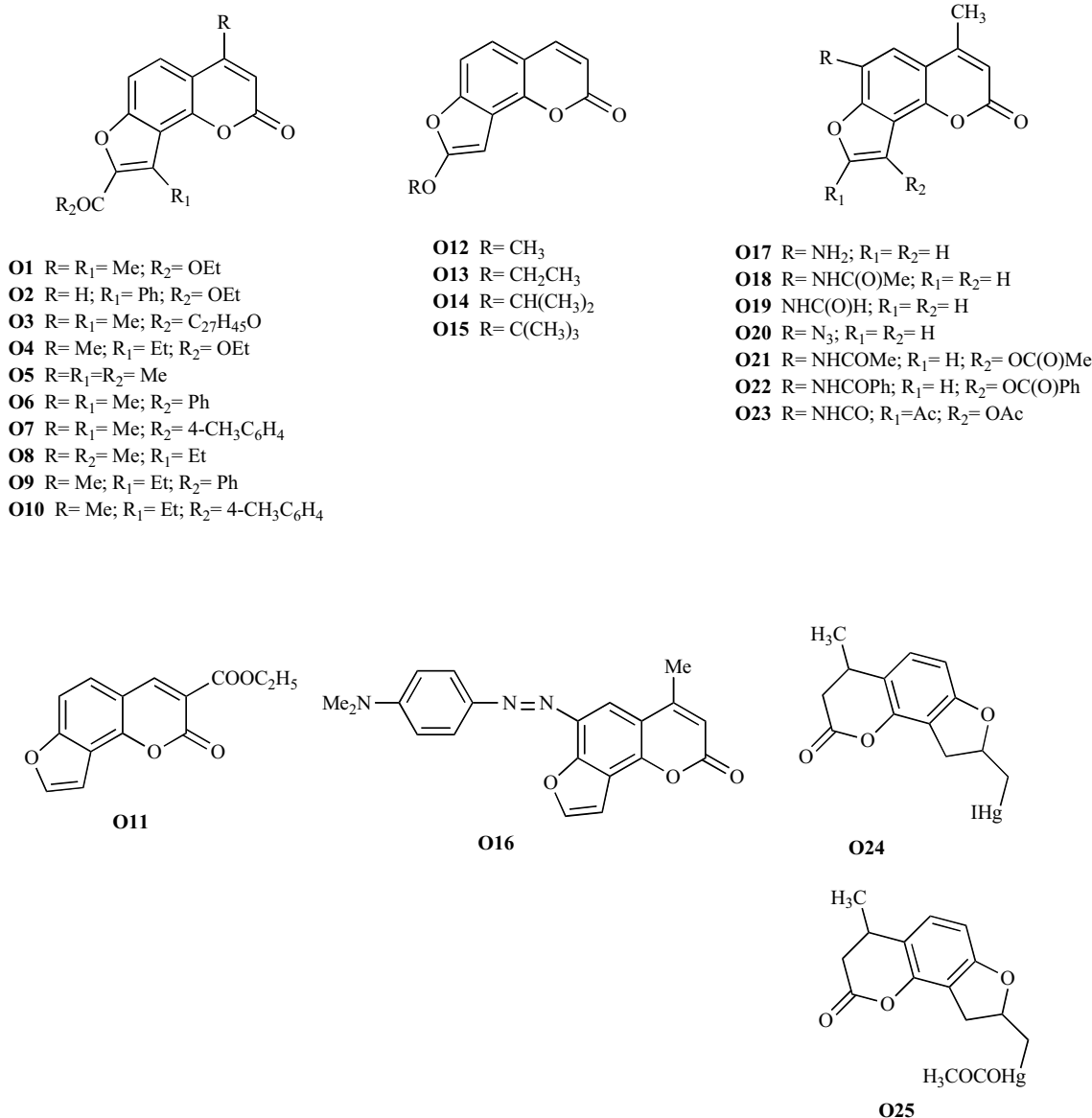


AA8



AA9

(Fig. (3). Contd....)

**Fig. (3).** Synthetic angular furanocoumarins.

site of angelicin (**A1**), in terms of extent of intercalation and DNA-photobinding, was studied [92]. The introduction of one methyl group both in the 3 or 4 and in 4' or 5' position increases the affinity of **A1** toward DNA for the molecular complex formation and enhances the DNA-photobinding, even if to a different extent. The increase is more pronounced for occupancy of 5' or 4' positions. The photoreactivity is also enhanced, but to a larger extent for 4',5'-dimethylangelicin (**M8**). No steric hindrance, therefore, seems to be exerted by the introduction of one or two methyl groups at level of the photoreactive sites of **A1**. The introduction of third methyl group in 4',5'-dimethyl (**M8**) or in 3,4-dimethylangelicin (**M7**) exhibits a strong enhancement of the DNA photobinding; in particular 4,4',5'-trimethylangelicin (**M9**) appears the most photoreactive towards DNA. Angelicins carrying groups in 3,4 positions exhibit lower antiproliferative activity than derivatives carrying methyl

groups in 4,5' positions. No correlation was observed between antiproliferative activity and DNA-photobinding; may be that the presence of methyl groups in 3,4 or in 4',5' positions affects the type of cycloadducts formed. The different ratio of adducts may affect the antiproliferative effect.

Conconi *et al.* [93] investigated the antiproliferative activity and phototoxicity of two methyl derivatives of 5-methoxyangelicin, 4,4'-dimethyl-5-methoxyangelicin (**M10**) and 3,4'-dimethyl-5-methoxyangelicin (**M11**). The first compound proved a promising candidate for use in clinical trials because of a high inhibitory effect on the growth of human cell lines coupled to low skin phototoxicity.

Cellular bioavailability of 8-methoxypsoralen (8-MOP), 4,6,4'-trimethylangelicin (TMA) (**M6**) and chlorpromazine (CPZ) was investigated *in vitro*, using low doses of UVA relevant for the clinical setting of extracorporeal photother-

apy (ECP) [94]. No intracellular fluorescence of **M6** could be observed, suggesting that this drug did not penetrate the cell membranes or, more likely, that it underwent rapid photobleaching inside the cells. Considering that **M6** was found to be the most efficient pro-apoptotic photosensitizer, while in a previous study **M6** was unable to induce immunosuppression in the animal model, it may hypothesize that apoptosis and immunosuppression in ECP are not interrelated. If apoptosis is a key factor that determines the efficacy of ECP, then **M6** may be a better photosensitizer than 8-MOP. Indeed, the apoptotic activity of **M6** has been found in this study to be higher than that of 8-MOP. On the other hand, 6,4,4'-TMA (**M6**), unlike 8-MOP and CPZ, does not induce immunosuppression in rats [95]. It cannot be ruled out that both apoptosis and immunosuppression are independent factors that play important role in ECP.

Despite strong evidence concerning the high efficiency of PUVA therapy (psoralen plus UVA light), its mechanism of action has not yet been fully elucidated. Recently Viola *et al.* [96] evaluated in a cell line of human keratinocytes (NCTC-2544) the effects of two linear psoralen derivatives, 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP), that are widely used in PUVA therapy and two angular derivatives, Angelicin (**A1**) and 6,4,4'-TMA (**M6**). All derivatives photoinduce cellular death, **M6** being the most active compound. The cell cycle analysis showed that the four derivatives induce, 24 h after irradiation, a cell cycle arrest in G1 phase later followed by massive apoptosis. The G1 arrest is correlated to an increase in the expression of p21^{Waf1/Cip1}, a protein associated with the cell cycle block and apoptosis. Furthermore, treatment of NCTC-2544 resulted in p53 activation by 5-MOP, 8-MOP, and **A1** but not **M6** and its phosphorylation at serine-15.

Angelicin Isosters

8-Methoxypsoralen (8-MOP), generally used in therapy, leads to short-term and long-term side effects, such as erythema and genotoxicity, respectively. These effects are associated with the induction of covalent adducts in DNA, mostly to inter-strand cross-links [97, 98]. In order to obtain more effective and less toxic drugs, several monofunctional derivatives, mostly angelicin analogues (iso-psoralens), have been prepared and studied. The angular structure of these compounds prevents, for geometrical reasons, the formation of inter-strand cross-links, and makes them less toxic than psoralens [91].

One important field of research is the synthesis of furanocoumarins isosters, such as azapsoralens [99, 100] and some sulfur and selenium derivatives [101], obtained through the insertion of a heteroatom into the furanocoumarin nucleus. Interesting angelicin isosters are also some furoquinolones, in which the oxygen at the pyrone ring is replaced by a nitrogen atom [102].

Thioangelicin Derivatives

Among the varied modifications reported in the angelicin molecule (**A1**), many analogues are characterized by the introduction of sulfur in place of oxygen in the pyrone ring of **A1**. This modification seems to give rise to a monofunctional psoralen with improved light adsorption, dark interaction and

photobinding with DNA. One possible synthesis of the thioangelicin could have made using 4-bromobenzofuran-5-carboxaldehyde obtained in several steps most of which gave only moderate to low yields, as described in Keil *et al.* [103]. A new convenient synthesis of thiopyrano[2,3-*e*]benzofuran (thioangelicin) (**S1**) was then realized by Jakobs *et al.* [104].

1-Thioangelicin (**S1**) was deeply studied with the aim to investigate the role of the substitution of sulfur for oxygen. The compound was examined by X-ray diffraction and its interaction with DNA was studied by means of linear flow dichroism, chromatography and ¹H-NMR. Steric and electronic features of 1-thioangelicin were studied also through theoretical calculations, including molecular mechanics optimizations, docking studies and frontier molecular orbital investigations. The experimental data indicated that thioangelicin (**S1**) is able to intercalate in the DNA helix and that subsequent irradiation yields a *cis-syn* adduct, in agreement with theoretical calculations [105].

The synthesis of thioisosters of angelicin (**A1**) was described just in 1984. Mosti *et al.* [106] synthesized a series of *N,N*-disubstituted 4-amino-3-chloro-2H-thieno[2,3-*h*]-1-benzopyran-2-ones, which are 7-thioisosteres of 4-amino-3-chloroangelicins. One of these compounds, the 3-chloro-4-morpholino-7-thioangelicin (**S2**), caused 70% of the antiproliferative effect of 8-MOP without inducing erythema on guinea pig skin. Subsequently, Mosti *et al.* [107] reported the synthesis of angelicin heteroanalogues in which the furan is replaced by thiophene (**S3**, **4**, **5**, **6**) and position 3 of the α -pyrone ring retains an electron-withdrawing group, as in 3-carboxyangelicin (**O11**). The antiproliferative activity of the thioangelicin was tested in different substrates and was higher than that of the natural parent compound angelicin (**A1**) and lower than that of 8-MOP. All the new heteroanalogues appeared to be free of the known phototoxicity of furocoumarins on the skin. The thioangelicin 2H-Thieno[2,3-*h*]-1-benzopyran-2-one (**S6**) induced strong inhibition of T2 bacteriophage infectivity and was able to repress the DNA synthesis in Ehrlich ascites cells and the clonal growth in HeLa cells (Figs. 4, 5).

Clarke *et al.* [108] developed a new route to the synthesis of 5'-substituted derivatives of 7-thioisopsoralens. They synthesized a novel 5'-substituted 7-thioisopsoralen (8-Methoxy-8,9-dihydro-2H-thieno[2,3-*h*]chromen-2-one) (**S7**) via a Claisen rearranged allyl aryl ether followed by reductive ozonolysis in the presence of a suitable solvent. Methods exist in the literature by which the alcohol moiety may be converted into a thiol. However it was shown that if the hydroxyl moiety is directly converted into the thiol, during the Claisen rearrangement the thiol reacts with the alkene to afford undesired products. Clarke *et al.* [108] utilised dimethylthiocarbonyl chloride, allowing the conversion of the alcohol moiety into a protected thiol. Subsequent deprotection could then be performed only after terminal alkene had been cleaved via ozonolysis.

With the aim of obtaining new furocoumarin derivatives, Jakobs *et al.* prepared some thio and seleno derivatives of psoralen [101]. Miolo *et al.* [109] showed that, in the series of thiopsoralens, the replacement of intracyclic oxygen with sulfur generally leads to increased DNA photobinding [110].

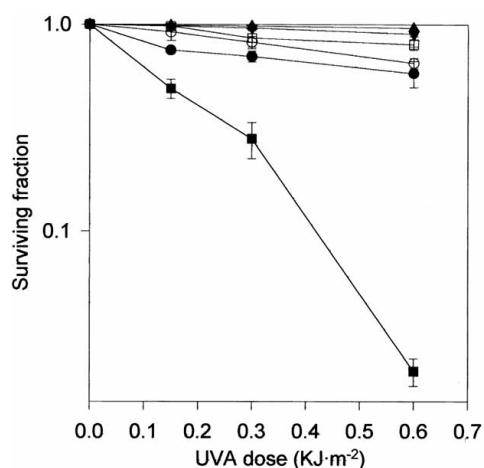


Fig. (4). T2 phage infectivity; virus particles were exposed to increasing UVA doses in the presence of thionangelicin derivatives (5 μM) and the numbers of the plaque forming units was determined. The symbols are: compound S3 (\blacktriangle); compound S4 (\blacktriangledown); compound S5 (\bullet); compound S6 (\blacksquare); 8-methoxypsoralen (\circ); angelicin (\square). The bars represent the standard errors.

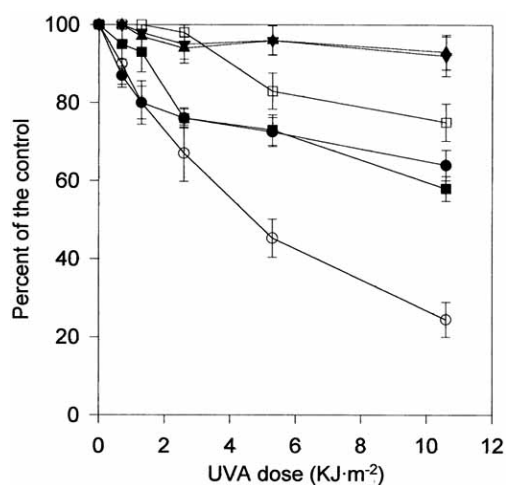


Fig. (5). Inhibition of DNA synthesis observed in Ehrlich cells. The tumor cells were incubated with tritiated thymidine and exposed to increasing UVA doses in the presence of thioangelicin derivatives (20 μM); then the acid insoluble radioactivity was determined. The symbols are: compound S3 (\blacktriangle); compound S4 (\blacktriangledown); compound S5 (\bullet); compound S6 (\blacksquare); 8-methoxypsoralen (\circ); angelicin (\square). The bars represent the standard errors.

With the aim of studying the effect of substituting oxygen by sulfur in the angular derivatives of psoralen, some new thioangelicins have been prepared [111].

Miolo *et al.* [109] studied two synthesized isomers of furocoumarins having a sulfur atom in their five-member ring, 4,4',5'-trimethyl-1'-thioangelicin (S8) and 4,6,4',5'-tetramethyl-1'-thioangelicin (S9), in terms of interactions with DNA, both in the ground state and after UVA absorption. The compounds were able to intercalate the macromolecule and to photobind efficiently, forming C4-cyclo-adducts with thymine. The antiproliferative effect of this binding was

shown in Ehrlich and HeLa cells and by T2 phage inactivation. Test on *Salmonella typhimurium* indicated low mutagenic activity. In particular S8 had photobiological activity comparable with that of 4,6,4'-trimethylangelicin (M6), but it was less mutagenic. Replacement of the oxygen atom by a sulfur (1'-thieno-4,6,4'-trimethylangelicin) (S10) increases the UV absorption of the M6 and its capacity to photobind to DNA *in vitro* but does not yield a comparable enhancement of its photosensitizing properties *in vivo*. This might be due to various reasons, for instance to an increase in the lipophilic character that could modify the behavior *in vivo* [112].

Pyrazolocoumarins

Mosti *et al.* [113] synthesized angelicin heteroanalogues in which the furan moiety is replaced by a 1-substituted pyrazole moiety (N1, 2, 3, 4) and position 3 of the α -pyrone ring retains an electron-withdrawing group, as in 3-carboxyangelicin (O11). These pyrazolocoumarins did not show effect upon UVA irradiation, but other biological activities were tested and one of these molecules showed good anti-inflammatory and antipyretic properties.

Furoquinolinones

With the aim of obtaining effective drugs for PUVA therapy, angelicin bioisosters named furoquinolinones were prepared and studied [102, 114]. Among them, 1,4,6,8-tetramethylfuro[2,3-*h*]quinolin-2(1H)-one (FQ) (N5), and 4,6,8,9-tetramethylfuro[2,3-*h*]quinolin-2(1H)-one (N6) are the more interesting. Both molecules demonstrate a strong photosensitizing activity, but N5 also shows skin phototoxicity and clastogenic activity [115] while these side effects are reduced or absent with N6 [116, 117].

A study about photobiological activities of N5 revealed that this angelicin isoster is characterized by a strong photosensitizing activity, higher than that of 8-MOP and 4,6,4'-trimethylangelicin (N6). Upon UVA irradiation N5 induces various types of lesions in mammalian cells in DNA: single-strand breaks, many monoadducts and covalent DNA-protein cross-links, but not interstrand cross-links. Thus, in spite of its higher capacity for damaging DNA, N5 showed a skin-phototoxicity potency very similar to 8-MOP [115].

Compound N5 showed a significant antiproliferative activity also in the dark, without UVA activation (Fig. 6). The cytotoxic activity of N5 in the dark was detected in HeLa cells and in normal human lymphocytes. This compound showed notable antiproliferative effects, barely lower in comparison with ellipticine, used as a reference. Similar results were obtained studying the N5 capacity for forming chromosome aberrations. N5 appeared to be much less genotoxic. The ability of N5 to damage DNA was investigated using alkaline elution and the formation of single-strand breaks and DNA-protein cross-links was observed. Moreover, experiments carried out with neutral elution showed the formation of double-strand breaks [118].

Substituting at 4 position the hydrophilic hydroxymethyl group for the hydrophobic methyl one, a new furoquinolinone was prepared, 4-hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1H)-one (N7). This compound showed high

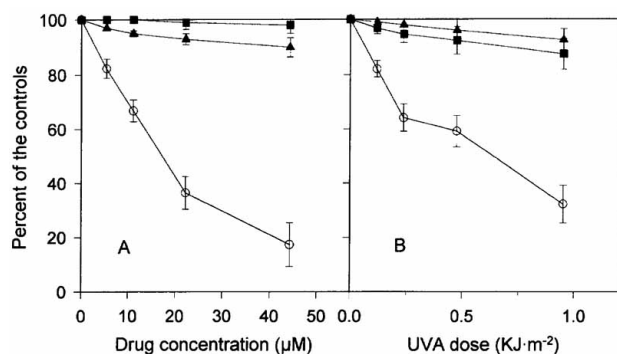


Fig. (6). Inhibition of DNA synthesis in Ehrlich ascites cells. Panel A. By incubation in the dark. Panel B. Upon UVA irradiation; in these experiments all compounds were assayed at 2 µM. The symbols are: N5: ○; 8-methoxypsoralen: ▲; M6: ■. The bars represent the standard errors.

antiproliferative activity on mammalian cells, due to its capacity to induce large amount of DNA-protein cross-links, without forming interstrand cross-links, considered mainly responsible for furanocoumarin genotoxicity [119].

Chilin *et al.* [120] reported a new synthesis of N7. This new profitable way allowed to synthesize also 4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1H)-one (N8), and 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1H)-one (N9). These molecules inhibited in the dark topoisomerase II, leading to a moderate antiproliferative activity in mammalian cells. The atiproliferative activity was also tested upon UVA irradiation in these cells and all compounds showed higher activity than 8-MOP without mutagenicity and skin phototoxicity, with the best results for N9 (Fig. 7).

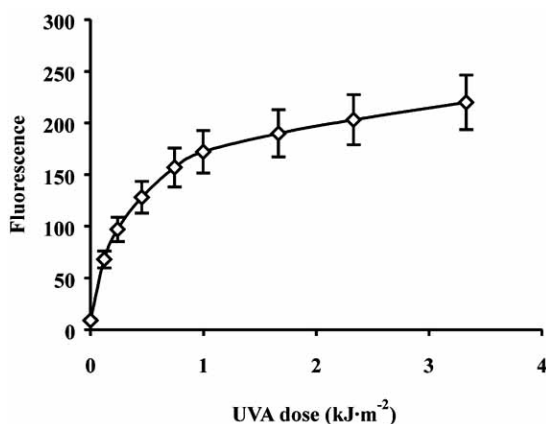


Fig. (7). Photobinding of N9 to calf thymus DNA *in vitro*; the irradiated DNA was precipitated, hydrolized and submitted to fluorescence determinations.

A series of furoquinolinones unsubstituted at the N₁ position (N10, 11, 12, 13, 14) was synthesized [121]. Photobi-

ological activities of these compounds were studied in comparison with N6 and 8-MOP. The anti-proliferative activity was tested upon UVA irradiation in mammalian cells, studying DNA synthesis and clonal growth capacity, and in micro-organism, evaluating T2 infectivity. Almost all compounds were more active than 8-MOP, and free of mutagenic activity and skin phototoxicity. Among them, N11 appeared to be the most effective one and a new potential drug for PUVA therapy and photopheresis (Fig. 8).

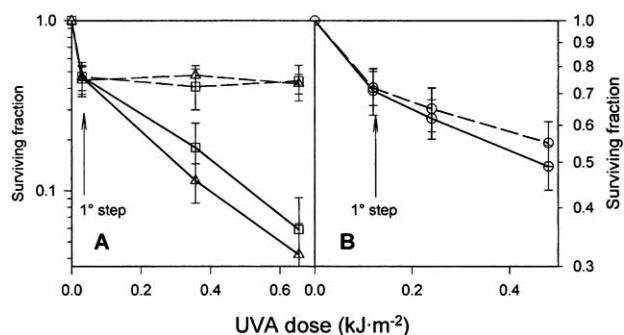


Fig. (8). Clonal growth of HeLa cells after sensitization with the double irradiation method. Cells were exposed to UVA light in the presence of N11 (2µM), and 8-methoxypsoralen (5 µM). The irradiated samples were washed with PBS (dotted lines) or not washed (solid lines), and then submitted to the second irradiation step performed with increasing UVA doses. Clonal growth capacity was determined. Symbols: N11, Δ; N6, □; 8-methoxypsoralen, ○. Panel A: first irradiation step: 0.03 kJ x m⁻²; Panel B: first irradiation step: 0.12 kJ x m⁻².

Among furoquinolinones prepared with the aim of moderating the strong toxic effects of 1,4,6,8-tetramethyl derivative (N5), an interesting derivate is 1,4,8-trimethylfuro[2,3-*h*]quinolin-2(1H)-one (N15). This compound showed a photobiological activity lower than that N5, but considerable higher than 8-MOP. Contrary to classic furanocoumarins, N15 induced a strong inhibition of protein synthesis in mammalian cells. Genotoxicity and skin erythema induction were virtually absent. Its low toxicity seems to be connected to a particular reaction mechanism: differently from furanocoumarin derivatives, N15 induces low levels of DNA-protein and no inter-strand cross links, but forms covalent RNA-protein linkages, lesions not observed with known furanocoumarins. Moreover, this analogue generates reactive oxygen species to a considerable extent [122].

Pirroloquinolinones

Recently, Barraja *et al.* [123] reported the synthesis of a new class of compounds, the pyrrolo[2,3-*h*]quinolinones, angelicin isosters in which both oxygen atoms are replaced with nitrogens. Pyrroloquinolinones N16-24 represent an interesting class of potentially useful compounds in photochemotherapy. They are isosters of the tricyclic system of angelicin (A1) with additional substituents in positions 3 and

7 that increase the conjugation of the system. The conjugation of the tricyclic ring with phenylsulfonyl groups strongly increases their photoactivity. Studies about the mode of actions of this series of compounds revealed that they do not intercalate DNA and do not induce photodamage to the macromolecule (Fig. 9). On the contrary, they induce significant photodamage to lipids and proteins. The lack of affinity of the new compounds with DNA upon UVA irradiation could be of great relevance in modulating the long-term toxic effect such as cancer of mutagenesis exhibited by psoralen.

Tetracyclic Benzpsoralen Analogues

One of the most promising strategies to obtain mono-functional furanocoumarins involves incorporating one of the reactive double bonds in a benzene nucleus forming benzofuranocoumarins. This approach results in molecules that have a high propensity for photoreaction with DNA and also helps to overcome some of the phototoxic effects usually shown by furanocoumarins [124, 125]. In order to increase the stability of the complex formed by the interaction of the molecule with DNA, Gonzalez-Gomez *et al.* [126] investigated the effects of introducing nitrogen atoms into the polycyclic skeleton. Pyridazino[3,4-*j*]angelicins (**T1-6**) and pyridazino [3,4-*h*]psoralens were prepared in good yield from resorcinol through a direct and generally applicable synthetic route.

The synthesis and the properties of tetracyclic psoralen analogues based on a dibenzofuran moiety was reported. Some of them showed a good ability to inhibit the *in vitro* growth of different human cell lines [127]. Recently, the same authors prepared the structurally related analogues **T7-11** [128]. Among the tested compounds, the angular pyranocarbazole **T7** showed the highest inhibitory activities *in vitro* against breast cancer (MCF-7), non-small cell lung cancer (NCI-H460) and central-nervous-system cancer (SF-268) cell lines. The strongly angular compounds **T7** and **T9** showed, in general, higher activity than linear compounds, even if the orientation of the rings and functional groups also seems to play a role.

Amino Derivatives

Among the furanocoumarins the amino derivatives of **A1** are the most useful for studying biological activities, since the high water-solubility provides active transport of a furanocoumarin salt in biological substrates and a more marked pharmacological effect. 4-Methylangelicins are obtained by condensing 5-aminomethylene-6,7-dihydrobenzofuran-4-one with dichloroacetic acid chloride in the presence of a tertiary amine into 3-chloro-3,4,5,6-tetrahydroangelicin with subsequent dehydrochlorination and aromatization [113]. Aminomethyl derivatives of angelicins are obtained by substituting the halogen of a halomethyl group of the appropriate an-

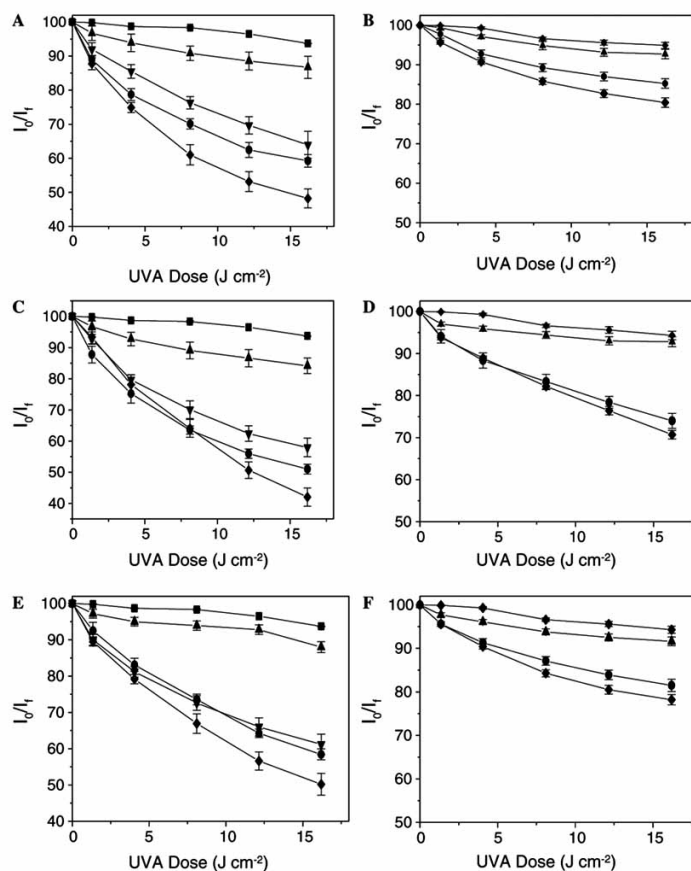


Fig. (9). Photosensitising effects on BSA (Panels A, C and E) and RNaseA (panels B, D and F) of compounds **N17** (A, B), **N16** (C, D), and **N23** (E, F), at the dose of 10 μ M after irradiation, in different conditions: in aerated (\bullet) and deaerated (\blacktriangle) solutions; plus SOD 2000 UI/ml (\blacktriangledown); in deuterated phosphate buffer (\diamond); (\blacksquare) BSA alone.

gelicin [129]. Studies about the biological activity of a series of aminoangelicins confirmed their use as photochemotherapeutic agents [129, 85]. Traven *et al.* [130, 131] reported the synthesis of 6-amino-4-methylangelicin (**AA1**) in which the amino group is on the benzene ring. Sakharuk *et al.* [132] reported some reactions which gave new heteroaromatic derivatives of 6-amino-4-methylangelicin, some pyrrolifuranocoumarins and 6-pyrazol-4-methylangelicin (**AA2-10**).

Other Angelicin Analogues

Furanocoumarins having electron-acceptor substituents, such as carbonyl-containing groups, in the furan or pyran ring, show low geno- and phototoxicity and seem to be promising molecules for photochemotherapy. Tolmachev *et al.* [133] developed a new procedure for the synthesis of 8-alkoxycarbonylangelicins by base-catalyzed cyclization of *ortho*-acyl(hydroxy)-coumarins with haloacetic acid esters. The procedure was successfully applied to prepare a series of new 8-alkoxycarbonyl- and 8-acylangelicins (**O1-10**).

In order to obtain new monofunctional drugs for photochemotherapy, Iester *et al.* [134] also synthesized a series of 3-acylangelicins and 3-alkoxycarbonylangelicins. These new compounds appeared to be free of the known phototoxicity of furanocoumarins on the skin and at a genetic level. Among them, an interesting analogue was 3-carbethoxyangelicin (**3-CA, O11**). A biological study of this compound showed that it does not photoreact with DNA but induces a moderate antiproliferative activity [135]. 3-CA (**O11**) proved to be extremely sensitive to ultraviolet A (UVA) light, undergoing rapid photolysis. One photolysis product was isolated and identified by Marzano *et al.* [136]. By means of alkaline elution, they observed that **O11** and its photolysis products are able to induce a large amount of single-strand breaks in DNA *in vivo*. The results obtained suggest that the photodynamic mechanism of action of **O11** results very likely from the capacity of the molecule and its photolysis products to produce singlet oxygen.

Among the several analogues of angelicins, various oxoisopsoralens were also synthesized. Clarke *et al.* [108] described the synthesis of a series of 5'-substituted 7-oxoisopsoralens (**O12-15**).

Traven *et al.* [137] reported the use of dihydrofuro[2,3-h]coumarin-9-one as a synthon in the synthesis of several 6-substituted angelicins. They obtained 6-aminocoumarin-9-one by the reduction of 6-phenylazo-4-methyldihydrofuro[2,3-h]-coumarin-9-one. The diazotization, *N*-acylation, and *N*-formylation of 6-aminocoumarin-9-one were realized. The diazo group at position 6 was substituted by chlorine atom and azide group and was reduced to hydrazino group. The synthesized 6-substituted 4-methyldihydrofuro[2,3-h]coumarin-9-ones were converted into the corresponding angelicins by reduction of the carbonyl group of the dihydrofuran ring and subsequent dehydration of the obtained alcohol and also by acylation with the fixed enolic form of the dihydrofuranone ring. These reactions gave five 6-substituted 4-methylangelicins (**O16-20**), and three 6-substituted 9-acetoxy-4-methylangelicins (**O21-23**).

Furanocoumarins are used in the treatment of epidermal proliferative disorders because of their ability to enter cells

and photo cross-link DNA. However, lipids and proteins, including a specific membrane receptor, are also potential target for these compounds. To better elucidate the site of action of furanocoumarins, Martey *et al.* [138] synthesized a series of 5'-mercurio-substituted derivatives of 4',5'-dihydro-psoralen (**O24, O25**). These compounds are identified by their heavy metal content and were used as a model to deliver thiol derivatives into keratinocytes.

CONCLUSION

The application of different psoralen molecules with potentially fewer acute side effects has been one of the most recent advancements in photochemotherapy. Psoralen-containing plants have been used for centuries in popular medicine to treat vitiligo, a skin disease characterized by lack of pigmentation. Further advancement in treatments using different psoralen molecules should strive to decrease the possibility of long-term side effects such as cutaneous malignancies. One of the directions for continued refinement of photochemotherapy in the future, as well as one of the new paradigms associated with photochemotherapy itself, is development of other psoralen molecules that do not form bifunctional adducts, which provide a basis for the DNA cross-linking. One such class of furanocoumarins is the methylangelicins (angular furanocoumarins) which only forms monofunctional adducts. There is clearly a theoretical basis that monofunctional adducts would less likely promote cutaneous malignancies as compared to bifunctional adducts.

Furanocoumarins are biologically active natural compounds found mainly in plants belonging to the Umbelliferae and Rutaceae such as celery (*Apium graveolens*), carrots (*Daucus carota*) and parsnips (*Pastinaca sativa*), but also in plants of Rutaceae, Apiaceae, Asteraceae, Fabaceae, Oleaceae, Moraceae and Thymeleaceae families. One of the most known natural angular furanocoumarins was angelicin isolated from different plant species such as *Angelica* species. Angelicin showed anti-inflammatory, antipyretic and cytotoxic activities against the human KB cell line and antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Cladosporium cucumerinum*. Sphondin, with a methoxy group at the C6 position and pimpinellin, having two methoxy groups at the C5 and C6 positions, showed a strong inhibitory effect on NO production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophage cells. Cnididin showed cytotoxic activity through cell-cycle inhibition blocking cells in the G1 phase. Recent study afforded to the isolation of two novel angular-type furanocoumarin glycosides, peucedanoside A and peucedanoside B, along with a known compound apterin, from the roots of *Peucedanum praeruptorum* (Umbelliferae).

In order to reduce the risks of PUVA treatment various methylangelicins were prepared. These compounds showed a high antiproliferative effect and reduced genotoxicity in comparison with 8-methoxypsoralen (8-MOP). Among the varied modifications reported in the angelicin molecule, many analogues are characterized by the introduction of sulfur in place of oxygen in the pyrone ring of angelicin. This modification give rise to a monofunctional psoralen with improved light adsorption, dark interaction and photobinding with DNA. Angelicin heteroanalogues, in which the furan

moiety was replaced by a 1-substituted pyrazole moiety and position 3 of the α -pyrone ring retains an electron-withdrawing group, as in 3-carbetoxyangelicin, showed good anti-inflammatory and antipyretic properties.

Angelicin bioisosters, named furoquinolinones, were prepared and studied with the aim of obtaining effective drugs for PUVA therapy, these molecules demonstrated a strong photosensitizing activity.

A series of furoquinolinones unsubstituted at the N₁ position were synthesized. Almost all compounds were more active than 8-MOP and free of mutagenic activity and skin phototoxicity. Recently it was reported the synthesis of a new class of compounds, the pyrrolo[2,3-*h*]quinolinones, angelicin isosters in which both oxygen atoms are replaced with nitrogens. Pyrroloquinolinones represent an interesting class of potentially useful compounds in photochemotherapy.

One of the most promising strategies to obtain monofunctional furanocoumarins involves incorporating one of the reactive double bonds in a benzene nucleus forming benzofurocoumarins. Some of them showed a good ability to inhibit the *in vitro* growth of different human cell lines.

Amongst the furanocoumarins the amino derivatives of angelicin are the most useful for studying biological activities, since the high water-solubility provides active transport of a furanocoumarin salt in biological substrates and a more marked pharmacological effect. Studies about the biological activity of a series of aminoangelicins confirmed their use as photochemotherapeutic agents.

Finally furanocoumarins having electron-acceptor substituents, such as carbonyl-containing groups, in the furan or pyran ring, show low geno- and phototoxicity and seem to be promising molecules for photochemotherapy.

LIST OF COMPOUNDS ABBREVIATIONS

Natural Furocoumarins

A1	=	Angelicin
A2	=	Archangelicin
A3	=	8(<i>S</i>),9(<i>R</i>)-9-angeloyloxy-8,9-dihydrooroselol
A4	=	Isobergaptin
A5	=	Sphondin
A6	=	Pimpinellin
A7	=	Cnididin
A8	=	Peucedanoside A
A9	=	Peucedanoside B
A10	=	Apterin

Methylangelicins

M1	=	5-methylangelicin
M2	=	4,5'-dimethylangelicins
M3	=	4'- <i>N,N</i> -dimethylaminoethoxymethyl-4,5'-dimethylangelicin

M4	=	4'-aminomethyl-4,5'-dimethylangelicin
M5	=	6,4'-DMA
M6	=	6,4,4'-TMA (6,4,4'-trimethylangelicin)
M7	=	3,4-dimethylangelicins
M8	=	4',5'-dimethylangelicins
M9	=	4,4',5'-trimethylangelicin
M10	=	4,4'-dimethyl-5-methoxyangelicin
M11	=	3,4'-dimethyl-5-methoxyangelicin

Sulfur Analogues

S1	=	thiopyrano[2,3- <i>e</i>]benzofuran (1-thioangelicin)
S2	=	3-chloro-4-morpholino-7-thioangelicin
S3; S4	=	esters of 2-oxo-2 <i>H</i> -thieno[2,3- <i>h</i>]-1-benzopyran-3-carboxylic acid
S5	=	2-Oxo-2 <i>H</i> -thieno[2,3- <i>h</i>]-1-benzopyran-3-carboxylic acid
S6	=	2 <i>H</i> -Thieno[2,3- <i>h</i>]-1-benzopyran-2-one
S7	=	8-Methoxy-8,9-dihydro-2 <i>H</i> -thieno[2,3- <i>h</i>]chromen-2-one
S8	=	4,4',5'-trimethyl-1'-thioangelicin
S9	=	4,6,4',5'-tetramethyl-1'-thioangelicin
S10	=	1'-thieno-4,6,4'.trimethylangelicin

Nitrogen Isosters

N1, 2, 3, 4	=	1-methyl and 1-phenyl substituted 5-oxo-5 <i>H</i> -pyran[2,3- <i>e</i>]indazol-6-carboxylic acid
N5	=	1,4,6,8-tetramethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N6	=	4,6,8,9-tetramethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N7	=	4-hydroxymethyl-6,8-dimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N8	=	4-methoxymethyl-1,6,8-trimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N9	=	4-hydroxymethyl-1,6,8-trimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N10	=	4,9-Dimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N11	=	4,6,9-Trimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N12	=	4,8,9-Trimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N13	=	4,6,8-Trimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N14	=	4,6,8,9-Tetramethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one
N15	=	1,4,8-trimethylfuro[2,3- <i>h</i>]-quinolin-2(1 <i>H</i>)-one

- N16 = 8-Methyl-7-phenyl-3-phenylsulfonyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N17 = 8-Methyl-7-phenyl-3-benzoyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N18 = 3-Cyano-8-methyl-7-phenyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N19 = 3-Ethoxycarbonyl-8-methyl-7-phenyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N20 = 7,8-Dimethyl-3-phenylsulfonyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N21 = 3-Benzoyl-7,8-dimethyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N22 = 7-Benzyl-8-methyl-3-phenylsulfonyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N23 = 3-Benzoyl-7-benzyl-1,5,6,7-tetrahydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one
- N24 = 4,8-Dimethyl-1,7-dihydro-2*H*-pyrrolo[2,3-*h*]-quinolin-2-one

Tetracyclic Benzoporalen Analogues

- T1 = 8-Methyl-1,4-bis(methoxycarbonyl)pyridazino[4,5-*j*]angelicin
- T2 = 8-Methyl-1-methoxycarbonyl-4-pyrrolidincarbonylpyridazino[4,5-*j*]angelicin
- T3 = 8-methyl-1,4-bis(pyrrolidincarbonyl)pyridazino[4,5-*j*]angelicin
- T4 = 8-Methyl-4-(dimethylaminoethylcarbamoyl)-1-methoxycarbonyl-pyridazino[4,5-*j*]angelicin
- T5 = 8-methyl-1,4-bis(dimethylaminoethylcarbamoyl)pyridazino[4,5-*j*]angelicin
- T6 = 8-Methyl-1-(dimethylaminoethylcarbamoyl)-4-pyrrolidincarbonyl-pyridazino[4,5-*j*]angelicin
- T7 = ethyl 3,11-dihydro-3-oxopyrano[3,2-*a*]carbazole-2-carboxylate
- T8 = ethyl 2,10-dihydro-2-oxopyrano[3,2-*b*]carbazole-3-carboxylate
- T9 = ethyl 3,12-dioxopyrano[3,2-*a*]xanthene-2-carboxylate
- T10 = ethyl 3,7-dioxopyrano[3,2-*c*]xanthene-2-carboxylate
- T11 = ethyl 2,6-dioxo-2*H*,6*H*-pyrano[3,2-*b*]xanthene-3-carboxylate

Amino Derivates

- AA1 = 6-amino-4-methylangelicin
- AA2 = 6-Acetamido-4-methylangelicin
- AA3 = 6-Formamido-4-methylangelicin

- AA4 = 4-Methyl-6-(4-toluenesulfonamido)-angelicin
- AA5 = 6-(4-Hydroxybenzylideneimino)-4-methylangelicin
- AA6 = 4-Methyl-6-(4-nitrobenzylidenehydrazino)angelicin
- AA7 = 6-(4-Hydroxybenzylidenehydrazino)-4-methylangelicin
- AA8 = 6-(1-Ethoxycarbonylethylidenehydrazino)-4-methylangelicin
- AA9 = 6-(2-Butylidenehydrazino)-4-methylangelicin
- AA10 = 6-(3,5-Dimethyl-1-pyrazolyl)-4-methylangelicin

Other Angelicin Analogues

- O1 = ethyl 4,9-dimethyl-2-oxo-2*H*-furo[2,3-*h*]-chromene-8-carboxylate
- O2 = ethyl 2-oxo-9-phenyl-2*H*-furo-[2,3-*h*]-chromene-8-carboxylate
- O3 = Cholesteryl 4,9-dimethyl-2-oxo-2*H*-furo[2,3-*h*]-chromene-8-carboxylate
- O4 = Ethyl 9-ethyl-4-methyl-2-oxo-2*H*-furo[2,3-*h*]-chromene-8-carboxylate
- O5 = 8-Acetyl-4,9-dimethyl-2*H*-furo[2,3-*h*]-chromen-2-one
- O6 = 8-Benzoyl-4,9-dimethyl-2*H*-furo[2,3-*h*]-chromen-2-one
- O7 = 4,9-Dimethyl-8-(4-toluoyl)-2*H*-furo[2,3-*h*]-chromen-2-one
- O8 = 8-Acetyl-9-ethyl-4-methyl-2*H*-furo[2,3-*h*]-chromen-2-one
- O9 = 8-Benzoyl-9-ethyl-4-methyl-2*H*-furo[2,3-*h*]-chromen-2-one
- O10 = 9-Ethyl-4-methyl-8-(4-toluoyl)-2*H*-furo[2,3-*h*]-chromen-2-one
- O11 = 3-carbethoxyangelicin (3-CA)
- O12 = 8-methoxy-8,9-dihydro-2*H*-furo[2,3-*h*]-chromen-2-one
- O13 = 8-ethoxy-8,9-dihydro-2*H*-furo[2,3-*h*]-chromen-2-one
- O14 = 8-isopropoxy-8,9-dihydro-2*H*-furo[2,3-*h*]-chromen-2-one
- O15 = 8-(*tert*-Butoxy)-8,9-dihydro-2*H*-furo[2,3-*h*]-chromen-2-one
- O16 = 6-(*p*-Dimethylaminophenylazo)-4-methylangelicin
- O17 = 6-Amino-4-methylangelicin
- O18 = 6-Acetamido-4-methylangelicin
- O19 = 6-Formamido-4-methylangelicin

- O20 = 6-Azido-4-methylangelicin
 O21 = 9-Acetoxy-6-acetamido-4-methylangelicin
 O22 = 9-Benzoyloxy-6-benzamido-4-methylangelicin
 O23 = 9-Acetoxy-8-acetyl-6-formamido-4-methylangelicin
 O24 = Iodomercurio-angelicin
 O25 = Acetylmercurio-angelicin

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