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Bakuchiol: A Retinol-Like Functional Compound, Modulating Multiple Retinol and Non-Retinol Targets

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Background

Bakuchiol (Figure 1.1; Phenol, 4-[1E, 3S]-3-ethenyl-3, 7-dimethyl-1, 6-octadienyl) was first isolated by Mehta et al. from the *Psoralea corylifolia* seed in 1973.[1] Absolute configuration of bakuchiol was established in the same year by Parakasarao et al.[2] Bakuchiol has one asymmetric center and is shown to possess (S)-chirality.[3] Mechanistically, both the 4-hydroxystyryl and terpenic moieties of the compound seem to be important for its bioactivity. Total synthesis was also accomplished in 1973.[4] Banerji and Chintalwar reported the biosynthesis of bakuchiol and established the pathway by using phenylalanine and mevalonic acid as substrates.[5,6]

Bakuchiol is mainly obtained from the seeds of the plant *Psoralea corylifolia,* which is widely used in Indian as well as in Chinese medicine to treat a variety of diseases.[7] Traditional medicine practitioners in India and China have utilized the plant for centuries. *Psoralea corylifolia* is known by a wide variety of names, suggesting its widespread use. For example, babchi, baguchi, babachi, Bakchi in Hindi and by many other names depending on the Indian languages; Ravoli in Sri Lanka; Boh-gol zhee in Korea; Buguzhi in Chinese.[7] A recent chapter on *P. corylifolia* describes its botany, phytochemistry, and ethnopharmacology, along with the various pharmacological activities of the plant.[8] Bakuchiol has also been isolated from other plants, such as *P. grandulosa*,[9,10] *P. drupaceae*,[11] *Ulmus davidiana*,[12] *Otholobium pubescens*,[13] *Piper longum*,[14] and *Aerva sangulnolenta* Blum.[15]

Structurally, bakuchiol (Figure 1.1) belongs to the family of meroterpenes. Meroterpenes are terpenes having an aromatic ring in the chemical structure. The term meroterpenoid was first applied by Cornforth, in 1968, to describe natural products of mixed biosynthetic origin which are partially derived from terpenoids.[16] They are typically derived from higher plants though they have also been obtained from fungi[17] as well as having been produced synthetically. Meroterpenes are also widely distributed in marine organisms. They are particularly abundant within brown algae, but other important sources include microorganisms and invertebrates.[18] Interestingly, the 4-hydroxystyryl functionality present in bakuchiol is also present in resveratrol (Figure 1.2).[19]

Bakuchiol possesses antioxidant,[20–23] anti-inflammatory[24,10,25,26,] anti-bacterial,[27] anti-tumor,[28,29] cytotoxic,[30] heptaprotective,[31] and caspase-3 dependent apoptosis[32] properties. The cytotoxicity of bakuchiol is mainly due to its DNA polymerase 1 inhibiting activity.[33] Although bakuchiol has shown many physiological properties and has been known since 1973, its first commercial use in topical applications did not occur until 2007 when it was introduced to the market under the trade name Sytenol® A by Sytheon Ltd. of Boonton, New Jersey. The focus of this chapter is twofold. The first is to show evidence of bakuchiol's functional resemblance to retinol (Figure 1.3). The second is to provide an overview of the important physiological and biological properties of bakuchiol as they relate to three key skin care applications—(1) preventative & restorative anti-aging; (2) anti-acne; and (3) skin lightening/even toning—and the mechanisms by which it provides these benefits. Additionally, this chapter has been extended to include a few key targets that may have applications beyond skin care, as well as provide an overview of bakuchiol's antibacterial properties.

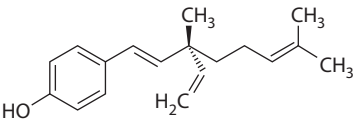

FIGURE 1.1 Structure of bakuchiol.

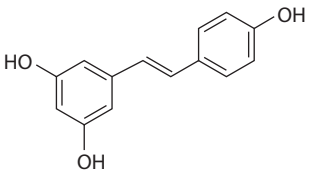

FIGURE 1.2 Structure of resveratrol.

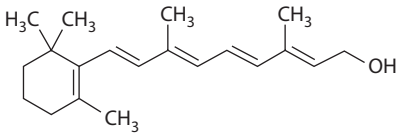

FIGURE 1.3 Structure of retinol.

Bakuchiol, a Functional Analog of Retinol

From the perspective of topically applied compositions, a small molecule that safely mimics the properties of retinol[34] (Figure 1.3) in reversing signs of aging, providing skin protection from sun-induced damage, providing solutions to problem skin, like acne and rosacea, and modulating pigmentation control, is a greatly sought after ingredient. Recently, Chaudhuri, using a simple comparative gene expression profiling of retinol and bakuchiol in a reconstituted full thickness skin substitute model, established a basis for making a claim that bakuchiol is a functional analog of retinol.[35] The journey from gene to protein is complex and tightly controlled within each cell. It consists of two major steps: transcription and translation. Together, transcription and translation are known as gene expression. Figure 1.4 illustrates the molecular signatures of retinol and bakuchiol through the volcano plot presentation of a DNA microarray experiment. The comparison of the volcano plots for bakuchiol (Figure 1.4a) and retinol (Figure 1.4b) shows similar overall shape, indicating similar overall modulation of gene expression in the skin substitute model. The effects of both compounds on specific pathways relevant to retinol functionality were then compared. First, a similar modulation of many (however, not all) genes coding for retinoid binding and metabolizing proteins was observed. A brief description of these genes as well as the impact of retinol and bakuchiol on each is presented in Table 1.1. Similarly, many genes involved in the generation and maintenance of the extracellular matrix (ECM) and the dermal-epidermal junction (DEJ) were similarly modulated by both retinol and bakuchiol.[36] Based on this and other data, Chaudhuri concluded that bakuchiol can function as a retinol-like compound through retinol-like regulation of gene expression.

Preventative and Restorative Anti-Aging

Targeting skin concerns early on can effectively prevent damage to the skin's surface and improve skin quality. Slowing down the aging process can be achieved by (i) antioxidant protection to limit direct oxidative damage to the cells, proteins, and DNA, (ii) controlling inflammation to minimize inflammation-induced skin damage, and (iii) use of sunscreen protection to prevent photodamage. The mechanisms and the sequence of events by which free radicals, the main culprit of oxidative damage, interfere with cellular

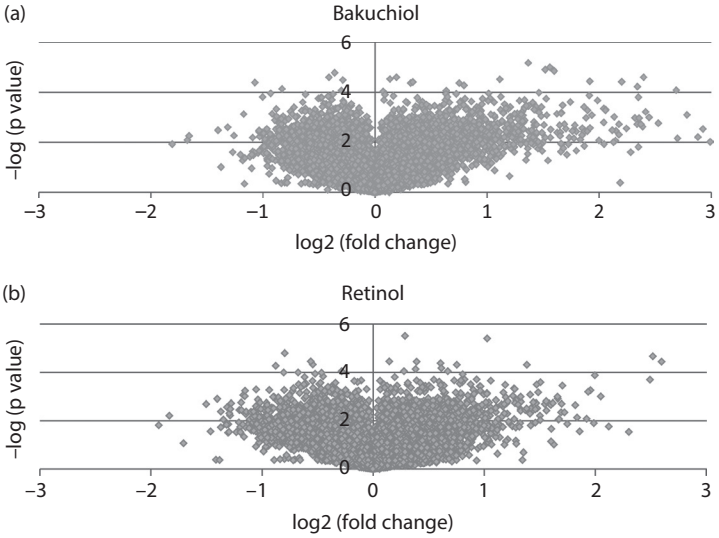

FIGURE 1.4 (a) Volcanic plot of DNA microarray data—Retinol. (b) Volcanic plot of DNA microarray data—Bakuchiol. (From Chaudhuri RK, Bojanowski K. *Int J Cosmet Sci* 2014;36(3):221–30. With permission.)

functions are not fully understood; but one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. This cellular damage causes a shift in the net charge of the cell, changing the osmotic pressure, leading to swelling and eventually cell death.[37]

Antioxidant

Multiple lines of compelling evidence substantiate the beneficial effects provided by the use of antioxidants. Direct application of antioxidants to skin has the added advantage of targeting antioxidants to those areas of the skin needing the protection most and, obviously, can easily be achieved. Topical application adds low molecular-weight antioxidants to the skin reservoir where they are available to protect the skin against oxidative stress. *Psoralea corylifolia* has a number of antioxidative components; bakuchiol is one of the most abundant and powerful antioxidants present in this plant.[21] Bakuchiol not only interferes with different free radical-producing systems, which are described below; but it also increases the function and effectiveness of endogenous antioxidants.

Haraguchi et al. have reported that bakuchiol inhibited NADPH-, ascorbate-, t-BuOOH-, and CCl(4)-induced lipid peroxidation in microsomes.[21] Indeed, bakuchiol was the most potent antioxidant in microsomes and its inhibition of oxygen consumption induced by lipid peroxidation was time-dependent. Bakuchiol also inhibits microsomal lipid peroxidation in a concentration-dependent manner showing 74.7% protection at a concentration of 10 μM. Bakuchiol also prevented NADH-dependent and ascorbate-induced mitochondrial lipid peroxidation. In view of its solubility in lipid and water (at higher pH), bakuchiol is expected to be distributed in both of these phases. This may account for its low $IC_{50} = 6.1 \pm 0.2$ μM value against lipid peroxidation.[20,38]

Bakuchiol has also been found to protect human red blood cells against oxidative hemolysis and to protect against oxidative stress-induced retinal damage. With respect to the latter, bakuchiol attenuated optic nerve crush (ONC)-induced up-regulation of apoptotic proteins, including cleaved poly ADP ribose polymerase (PARP), cleaved caspase-3, and cleaved caspase-9.[39] Bakuchiol also significantly inhibited translocation of mitochondrial apoptosis induced factor (AIF) into the nuclear fraction and release of mitochondrial cytochrome c into the cytosol.

In validation of the foregoing effect, Chaudhuri and Marchio have recently shown that bakuchiol has broad-spectrum antioxidant activity (*in vitro*) and effectively quenches superoxide-, hydroxy-, peroxy-, peroxynitrile radicals, and singlet oxygen non-radical in addition to inhibiting lipid peroxidation.[23] As

TABLE 1.1

Fold Change in the DNA Microarray Experiment, and Role of Modulated Retinoid Binding and Metabolizing Genes (R: retinol; B: bakuchiol)

Gene	Full Name	Function and Comments
CRBP I; CRBP II; CRBP IV	Cellular retinol binding protein I, II and IV	CRBP I:R = 2.6; B = 4.2 CRBP II: R = NS; B = 4.1 CRBP IV: R = NS; B = 3.1 CRBP I mediates the cellular uptake of retinol, solubilizes and detoxifies it for further transport within the cytoplasm, and presents it to the appropriate enzymes to biosynthesize retinoic acid.
N6AMT2	N-6 adenine-specific DNA methyltransferase 2	R = NS; B = –2.1 Retinoic acid resistance might be overcome by the use of epigenetic modifying agents such as DNA methyl transferase inhibitors. Down-regulation provided by bakuchiol may reduce retinoic acid-induced toxicity.
TIG1	Tazarotene-inducible gene 1	R = 13.2; B = 12.9 Retinoid acid (RA) receptor-responsive gene. The expression of this gene is found to be down-regulated in a variety of human cancers as well as in acne, rosacea, and psoriasis. Up-regulation by bakuchiol may provide a solution to problem skin. Anti-acne clinical study results of bakuchiol has recently been reported (40).
DHRS9	Dehydrogenase/ reductase SDR family member 9 precursor	R = 5.5; B = 11.6 DHRS9 is involved in converting retinol to retinal and then to retinoic acid, the rate-limiting step for the biosynthesis of retinoic acid.
RETSAT	All-trans-13,14-dihydroretinol saturase	R = –2.9; B = –2.8 RETSAT expression is involved in adipocyte differentiation.
LRAT	Lecithin-retinol acyltransferase	R = 12.3; B = 82.2 Retinol esterification with long-chain fatty acid by LRAT is the key step in both absorption and storage of retinol.
CYP1A1; CYP1A2	Cytochrome P450	CYP1A1: R = 4.0; B = 4.9 CYP1A2: R = 3.6; B = 6.7 In addition to retinol dehydrogenase, P450s 1A1 and 1A2 genes are the major human P450s that catalyze the reaction of retinol to retinal.
RARB; RARG	Retinoic acid receptor beta -1; Retinoic acid receptor gamma -1	RARB: R = 5.6; B = NS RARG: R = 1.8; B = NS The actions of retinoids are generally mediated by the retinoic acid receptors (RARs alpha, beta, and gamma) and the retinoid X receptors (RXRs alpha, beta, and gamma). Both RARB and RARG are up-regulated, as expected, by retinol but not with bakuchiol.

presented in Table 1.2, its antioxidant profile, especially with respect to lipid peroxidation inhibitory activity, is far superior to natural tocopherol, a common topical antioxidant. Bakuchiol was found to be 60-fold more effective in inhibiting squalene than natural tocopherol (IC_{50} for bakuchiol 0.5 µg/mL vs. natural tocopherol 30 µg/mL). Squalene is particularly prone to photooxidation during sun exposure.[40] Hence, bakuchiol is expected to protect squalene and other skin lipids from oxidation due to its excellent lipid peroxidation inhibitory activity.

The protective activity of bakuchiol against oxidative damage to lipids and proteins has been investigated and rationalized based on the scavenging activity of bakuchiol against various oxidizing radicals including Cl(3)CO(2)(*), linoleic acid peroxyl radicals, LOO(*), DPPH radicals, (*)OH, and glutathiyl radicals by Adhikari et al.[20] The rate constants of the scavenging reactions, the transients formed in these reactions, and their mechanistic pathways have been probed using an optical pulse radiolysis technique. The methyl ether derivative of bakuchiol was also shown to prevent lipid peroxidation in rat brain homogenate, indicating participation of the terpenoid chain in scavenging LOO(*). In their study,

TABLE 1.2

Antioxidant Profile of Bakuchiol and Natural Tocopherol

Unit[a]	Peroxyl	Hydroxyl	Superoxide	Peroxynitrite	Singlet Oxygen	Lipid Peroxidation[b]
Bakuchiol	15,165	569	204	130	1,325	0.5
Tocopherol natural	813	Not detected	Not detected	1	1,110	30

[a] µmole Trolox equivalent/g.

[b] Squalene was used as a substrate for lipid peroxidation inhibitory activity; data is expressed in IC_{50} in µg/mL.

Adhikari et al. were able to demonstrate that the allylic radical formed initially was transformed into the phenoxyl radical at a later stage. These findings revealed the importance of the terpenoid moiety of bakuchiol in controlling its antioxidant action via radical scavenging.

Many studies have established that oxidative stress and mitochondrial dysfunction are two central factors contributing to the aging process. Bakuchiol was shown by Haraguchi et al. to be very effective in protecting mitochondrial functions against oxidative stress.[22] As noted earlier, bakuchiol prevented mitochondrial lipid peroxidation, inhibiting oxygen consumption originating in lipid peroxidation, in a time-dependent manner. Bakuchiol was also found to protect mitochondrial respiratory enzyme activities against both NADPH-dependent and dihydroxyfumarate-induced peroxidation injury.

ATP generation is an essential function in mitochondria. Recently, Seo et al. examined the effect of *Psoralea corylifolia* seed (PCS) extract on ATP synthesis. They found that both PCS extract and bakuchiol increased ATP synthesis in the hepatocytes of old mice whose ATP synthesis had been reduced by H_2O_2 treatment. Seo et al. further examined the impact of PCS extract on the integrity of the mitochondrial membrane structure which, according to Tsujimoto and Shimizu,[41] is involved in ATP energy production and mitochondrial function. According to their findings, PCS extract treatment led to a recovery in the mitochondrial membrane potential whose reduction had been induced by oxidative stress, evidencing a stimulation of mitochondrial respiration and restoration of mitochondrial energy metabolism.[42] These authors were also able to demonstrate that PCS extract and bakuchiol guarded against mitochondrial genome damage.

Another possible mechanism by which bakuchiol acts in addressing oxidative damage and stress is through interaction with various enzyme systems, especially those associated with the endogenous antioxidant defense system. Efficacy may, at least in part, manifest from a two pronged effort involving both radical scavenging and an interaction with enzyme functions. As presented in Table 1.3, in a side-by-side comparison with retinol, bakuchiol has been shown to stimulate the endogenous antioxidant defense system using a reconstituted full thickness skin substitute model. As indicated, with one exception,

TABLE 1.3

Gene Expression Profile of Bakuchiol and Retinol Related to Endogenous Antioxidant System

			Fold Change vs. Control	
Gene	Gene Description	Function	Retinol	Bakuchiol
GPX3	Glutathione peroxidase 3 precursor/extracellular glutathione peroxidase	Protect organism from oxidative damage. Reduce lipid hydroperoxides → alcohols and hydrogen peroxide → water	+2.5	+3.2
GSTT1	Glutathione S-transferase theta −1	Involved in the detoxification of endogenous compounds, such as peroxidized lipids, as well as the metabolism of xenobiotics.	+2.9	+3.0
GSTP1	Glutathione S-transferase P 1	Same as above	+2.8	+3.0
NQO1	NAD(P)H dehydrogenase [quinone]	This protein's enzymatic activity prevents the one electron reduction of quinones that results in the production of radical species	No effect	+5.0

bakuchiol and retinol showed a remarkably similar gene expression pattern with a very high statistical significance ($p \leq 0.05$). The only exception was that retinol had no effect on the NQO1 gene whereas bakuchiol had a fivefold stimulatory effect. NAD(P)H:quinone oxidoreductase 1 (NQO1) is a cytosolic protein that catalyzes metabolic detoxification of quinones, thereby protecting cells against quinone-induced oxidative stress, cytotoxicity, and mutagenicity.[43]

Inflammation

Skin aging and inflammation are critically linked. Enzymes associated with inflammation and the inflammatory responses, particularly chronic inflammation, are known to accelerate skin aging and degradation. Among the enzymes that synthesize pro-inflammatory mediators from the arachidonic acid pathway are the cyclo- and lipo-oxygenases.[44] Bakuchiol has moderate inhibitory activities against both 5-lipooxygenase (IC_{50} 23.5 μM)[24] and cyclooxygenase-1 and -2 (IC_{50} 14.7 and 514 μg/mL).[23] Studies have revealed that bakuchiol is a weak inhibitor of secretory and intracellular phospholipase A2 (PLA2) but dose-dependently reduced the formation of leukotirene B4 (LTB4) and thrombaxane B2 (TXB2) by human neutrophils and platelet microsomes, respectively.[24] Additionally, bakuchiol inhibited degranulation in human neutrophils, whereas superoxide generation was not affected. In mice, bakuchiol decreased cell migration, myeloperoxidase activity, and eicosanoid levels in the air pouch inflammation induced by zymosan. Applied topically, bakuchiol was also found to be effective as an inhibitor of edema and myeloperoxidase activity in the 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced ear edema and significantly reduced the PGE2 content and ear edema in the arachidonic acid-induced response. Bakuchiol is a natural anti-inflammatory agent that, among others, is able to control leukocytic functions such as eicosanoid production, migration, and degranulation in the inflammatory site. Inhibitory effects of bakuchiol in pro-inflammatory arachidonic acid pathway are summarized in Figure 1.5.

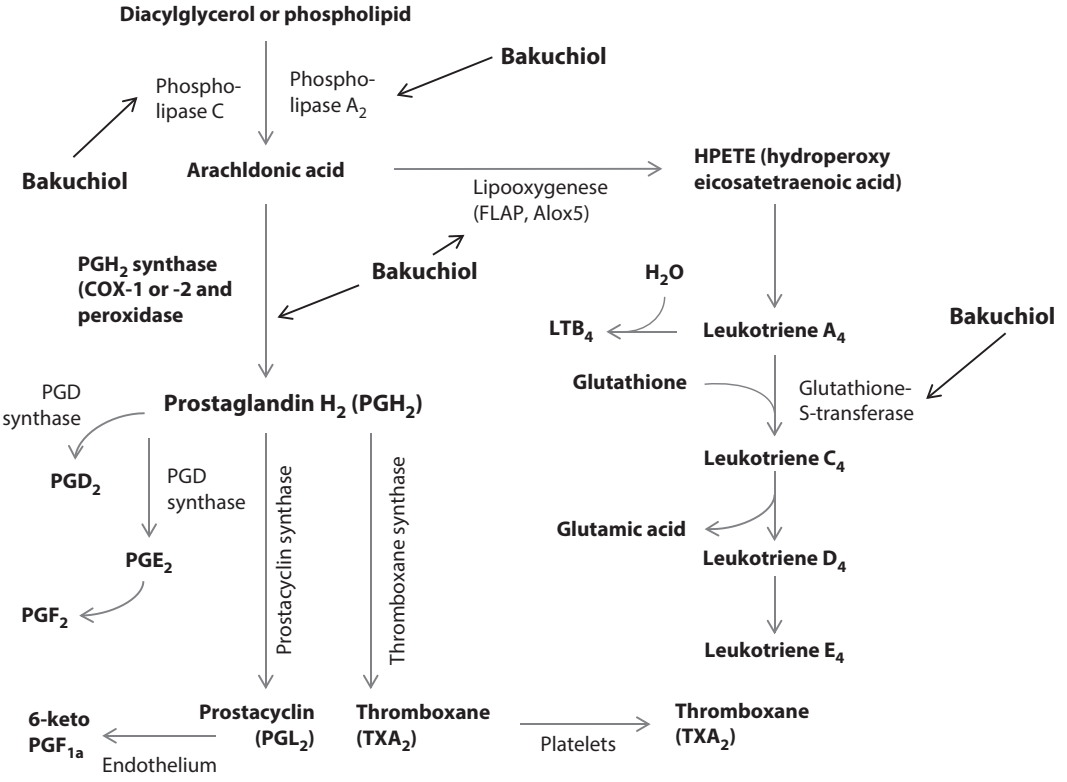

FIGURE 1.5 Bakuchiol inhibits multiple sites in pro-inflammatory arachidonic acid pathway.

TABLE 1.4

Pro-Inflammatory Gene Modulation by Bakuchiol and Retinol

Gene	Gene Description	Function	Fold Change vs. Control	
			Bakuchiol	**Retinol**
COX-1/PTGS1	Cyclooxygenase-1 (prostaglandin G/H synthase precursor)	Prostaglandin biosynthesis; acts as both a dioxygenase and a peroxidase	−3.6	−3.4
PLAA	Phospholipase A-2-activating protein	PLAA releases fatty acids from the second carbon group of glycerol. Upon downstream modification by cyclooxygenases, arachidonic acid is modified into eicosanoids. Eicosanoids include prostaglandins and leukotrienes, which are categorized as inflammatory mediators.	−7.7	No effect
PLA2G4A	Cytosolic phospholipase A2	Catalyzes hydrolysis of membrane phospholipids to release arachidonic acid, which is subsequently metabolized into eicosanoids	−2.6	−3.1
PTGER2	Prostaglandin E2 receptor EP2 subtype	Inflammatory reaction via the EP2 receptor through its regulation of TNF-alpha and IL-6	−2.4	−2.2
PTGER4	Prostaglandin E2 receptor EP4 subtype	Inflammatory reaction via the EP4 receptor through its regulation of TNF-alpha and IL-6	−6.1	−3.0
HPGD/15PGDH	15-Hydroxy prostaglandin dehydrogenase	HPGD is a catabolic enzyme controlling the biological activities of prostaglandins by converting them into inactive keto-metabolites	+21.8	+4.1

Validating the anti-inflammatory effect of bakuchiol, Chaudhuri conducted comparative gene expression profiles of bakuchiol and retinol on several pro-inflammatory genes using a reconstituted full thickness skin substitute model. As presented in Table 1.4, with the exception of two genes, phospholipase A-2-activating protein (PLAA) and 15-hydroxy prostaglandin dehydrogenase (HPGD) (15-PGDH), bakuchiol and retinol showed remarkable similarity in the down-regulation of the inflammatory genes. In the case of PLAA, bakuchiol produced a sevenfold down-regulation whereas retinol had no effect on PLAA. With HPGD, bakuchiol showed a 22-fold up-regulation in comparison to retinol's fourfold up-regulation. The HPGD gene encodes the enzyme HPDG, a member of the short-chain non-metalloenzyme alcohol dehydrogenase protein family, which is a catabolic enzyme controlling the biological activities of prostaglandins by converting them into inactive keto-metabolites. Reduced expression of HPGD contributes to the elevated levels of prostaglandins found in the skin following UVR exposure as demonstrated by Judson et al.[45] Following on their findings, these authors speculated that agents which prevent UVR-mediated down-regulation of HPGD could affect the acute or the long-term consequences of UVR exposure, including nonmelanoma skin cancer.

Erythema, a common form of inflammation, is the most obvious clinical sign of UV radiation exposure and becomes readily apparent within 6 h or less of UV exposure and is maximal at about 24 h.[46] COX dependent prostaglandin E_2 (PGE_2) is believed to be one of the mediators of UVR-induced erythema. Phospholipase A_2 (PLA_2), whose synthesis occurs only when skin is exposed to UV doses sufficient to cause erythema, is considered a rate limiting step in the generation of leukotrienes and prostaglandins. Hence, the two are intertwined in regards to erythema and their impact thereon.

Building upon the results attained in the above-mentioned investigation of the impact of bakuchiol and retinol on inflammation-related gene expression, Chaudhuri conducted a clinical study (unpublished) to assess the skin protection property of bakuchiol against erythema.[47] In this study, Chaudhuri determined the average L-, a-, and ITA (Individual Typology Angle) values of treated (with

TABLE 1.5

Reduction in Erythema Using 1% Bakuchiol Lotion

	Pre-Irr	Post-Irr	Δ L or ΔITA Value or Δa-Value
L-value (with bakuchiol)	65.69	66.25	–0.56
L-value (without bakuchiol)	66.45	60.71	–5.74 (Statistically significant $p < 0.001$)
ITA (with bakuchiol)	43.97	46.83	+2.86
ITA (without bakuchiol)	46.05	36.76	–9.29 (Statistically significant $p < 0.001$)
a-value (with bakuchiol)	8.53	8.38	–0.15
a-value (without bakuchiol)	8.17	16.32	+8.15 (Statistically significant $p < 0.001$)

a 1% bakuchiol lotion) and untreated skin of 10 human volunteers prior to irradiation/UV exposure ("Pre-Irr") and following irradiation/UV exposure ("Post-Irr"). As presented in Table 1.5, the results clearly showed a marked reduction in the manifestation of erythema, as evidenced by the significant difference in the delta or change in the L-, a-, and ITA values in those areas that were treated with the bakuchiol containing lotion as compared to the untreated areas.

More recently the role of nitric oxide (NO) as a contributor to the UV erythema response has been established.[48] NO is produced in the skin by NO synthase that can combine with superoxide to form peroxynitrite, a highly reactive oxidant and mediator of tissue injury. Similarly, large amounts of nitric oxide (NO) production following the induction of an inducible NO synthase (iNOS) gene has also been implicated in the pathogenesis of various inflammatory diseases. Bakuchiol has been shown to inhibit NO production in RAW 264.7 macrophages activated with interferon-γ and lipopolysaccharide. The mechanistic studies showed that bakuchiol inhibited the expression of iNOS mRNA through the inactivation of NF-κB.[25] Thus, bakuchiol is also expected to protect skin from UV induced erythema as well as from damage due to sun-induced iNOS gene over-expression.

Matrix Metalloprotease (MMP)

A major characteristic of aged and prematurely aged skin is a high degree of fragmentation of the dermal collagen matrix.[49] MMPs play a major role in protein and collagen degradation, which affects the structural integrity of the dermis. In normal skin, its production is in balance with their natural inhibitors tissue inhibitors of metalloproteinases (TIMPs); however, UV light is reported to enhance the synthesis of MMP in human skin *in vivo* leading to MMP-mediated collagen destruction. Sun exposure, especially substantial sun exposure, leads to an imbalance between the active enzymes, the MMPs, and their natural inhibitors (TIMPs) resulting in the accelerated destruction of connective tissues[50] and photoaging.[50] Therefore, protection of extracellular matrix proteins, such as collagens, in aged or photoaged human skin by the reduction of MMPs would be expected to retard the clinical manifestations of skin aging.

In this regard, it is well documented that retinol treatment (human clinical) reduces matrix metalloprotease expression and stimulates collagen synthesis in naturally aged, sun-protected skin and, perhaps more importantly, in photodamaged skin.[51] Given the many similar targets of retinol and bakuchiol, Chaudhuri compared the performance of bakuchiol and retinol on two key matrix metalloproteases, MMP-1 and MMP-12. As presented in Table 1.6, bakuchiol has a significant inhibitory effect on MMP-1

TABLE 1.6

Matrix Metalloprotease Inhibitory Activity of Bakuchiol and Retinol

Matrix Metalloprotease	Methods Used	Bakuchiol	Retinol
MMP-1 (collagenase)	Enzcheck collagenase assay kit (molecular probe)	50% inhibition at 1 mg/mL	Not determined
MMP-12 (elastase)	Calbiochem human neutrophile elastase kit (Cat # 324681)	70% inhibition at 1 μg/mL	8% inhibition at 1 μg/mL

and a markedly stronger inhibitory effect on MMP-12, far exceeding the effect of retinol (Table 1.6). Thus, based on retinol's known effectiveness and these results, it is expected that bakuchiol will provide an even stronger protection to the extracellular matrix proteins *in vivo.*

Extracellular Matrix Proteins

Emerging evidence indicates that intrinsic, chronological aging of the skin shares several mechanistic features with photoaging.[48] For example, collagen fragmentation is responsible for the loss of structural integrity and the impairment of fibroblast function in aged as well as photoaged human skin. In aged skin, collapsed fibroblasts produce low levels of collagen and high levels of collagen-degrading enzymes. This imbalance advances the aging process in a self-perpetuating, never-ending deleterious cycle. Treatments that stimulate production of new, non-fragmented collagen are, therefore, expected to provide substantial improvement in the appearance, health, and integrity of aged skin. Indeed, treatments such as topical retinol or retinoic acid have been clinically proven to stimulate production of new, undamaged collagen.[49] The attachment of fibroblasts to this new collagen allows stretch, which in turn balances collagen production and degradation, thereby slowing, if not reversing, the aging process.

Numerous studies have shown the restorative effects of topical application of all-*trans* retinoic acid (RA) on aging skin, including the partial restoration of collagens I, III,[47] and VII[52] and the restoration of the fibrillin-rich microfibrillar network.[53] These extracellular matrix (ECM) changing together with reduced MMP expression may, in part, explain the clinical improvement of photoaged skin produced by topical retinoids. In light of the similarities in targets of retinol and bakuchiol, one may also expect similar performance of bakuchiol in this regard as well.

In an effort to validate their DNA microarray analysis of the comparative effects of bakuchiol and retinol on collagen stimulation, Chaudhuri and Bojanowski measured collagen stimulation by ELISA and histochemistry methods. The ELISA assessment employed cell-culture conditioned media from neonatal (type I and IV collagens) or mature (type III collagen) fibroblasts.[36] Their findings, as summarized in Table 1.7, not only confirmed the up-regulation of types I and IV collagen in the DNA microarray study and the stimulation of type III collagen in the mature fibroblast model, but also demonstrated a significant improvement in collagen stimulation as compared to retinol. Hence, even greater restorative properties may be found with bakuchiol.

Skin Hydration and Barrier Homeostasis

Water homeostasis of the epidermis is essential for the normal function of the skin and for normal stratum corneum (SC) hydration. Dehydration of the SC is a typical characteristic of skin aging, especially in photoaged skin, and of many diseases associated with dry skin.[54] Water homeostasis is a determinant of skin appearance, mechanical properties, barrier function, and metabolism. In addition, it is indispensable in maintaining proper water balance of the body itself. One of the key genes associated with skin hydration and barrier homeostasis is CDH1, epidermal cadherin. Epidermal cadherin (E-cadherin) is essential for water barrier formation and is required for correct tight junction formation. Loss of E-cadherin in the epidermis *in vivo* results in prenatal death of mice due to the inability to retain a functional epidermal water barrier. E-cadherin regulates claudin-1, claudin-4, and ZO-1 localization by activating aPKC, which is implicated in tight junction formation and is considered to be a key protein for maintaining skin homeostasis.[55] Using EpiDermFT skin substitute, Chaudhuri and Bojanowski[36] have shown that both retinol and bakuchiol increased expression of CDH1 as well as AQP3, another

TABLE 1.7

Comparative Collagen Stimulatory Effects of Bakuchiol and Retinol

Test Material (10 µg/mL)	Collagen I	Collagen III	Collagen IV
Bakuchiol	147	150	119
Retinol	119	148	100

TABLE 1.8

Gene Expression Profile of Bakuchiol and Retinol Related to Skin Hydration and Barrier Homeostasis

			Fold Change vs. Control	
Gene	**Gene Description**	**Function**	**Bakuchiol**	**Retinol**
AQP3	Aquaporin 3	Aquaporin 3 is the water/glycerol transporting channel protein expressed in the epidermis which helps maintain the right level of skin hydration, elasticity, and barrier recovery.	4.3	3.5
CDH1	E-cadherin	Essential for water barrier formation and is required for correct tight junction formation	21.6	9.4

gene associated with water transport and whose expression is decreased during aging.[56] As indicated in Table 1.8, while the two had similar effects on AQP3, bakuchiol produced a marked increase in the gene expression of E-cadherin.

Following on the gene expression study, Chaudhuri and Bojanowski also conducted a clinical study demonstrating the anti-aging efficacy of a topical composition containing just 0.5% bakuchiol.[36] In that study, the composition was applied to the face of 17 healthy female subjects ranging in age from 40 to 65 years and who showed outward evidence of photoaging, including wrinkles, sagging, spots, and a dull complexion on the face, twice a day for 12 weeks. (One subject was removed from the study due to protocol violation.) Each subject's facial skin was evaluated through self-assessment, clinical grading, and instrument measurements, over the course of the treatment to assess any changes in the appearance of fine lines and wrinkles, elasticity, firmness, even toning, and overall signs of photodamage. Although some improvement was noted in most of the parameters after just four weeks, significantly more improvement was noted after the eighth week. These improvements continued to increase, even faster through the twelfth week of product application, indicating, perhaps, a certain degree of cumulative beneficial effect over time. These results were consistent amongst all three evaluation methodologies employed. Additionally, these results provided the ultimate validation of the *in vitro* results noted previously and were in line with the retinoid-type functionality of bakuchiol.

Anti-Acne

Acne is a complex, chronic, and common skin disorder of pilosebaceous units. There are four major targets presently governing acne therapy as follows: correcting the altered pattern of follicular keratinization; decreasing sebaceous gland activity; decreasing the follicular bacterial population, especially *P. acnes*; and producing an anti-inflammatory effect by inhibiting the production of extracellular inflammatory products through the inhibition of these microorganisms.[57] Dihydrotesterone (DHT) is not only involved in sebum production but also involved in the production of pro-inflammatory cytokines in acne.[58] In recent years there has been an increasing focus on the extent to which oxidative stress is involved in the pathophysiology of acne. Emerging studies have shown that patients with acne are under increased cutaneous and systemic oxidative stress. Indeed, there are indications that lipid peroxidation itself triggers the inflammatory cascade in acne.[59]

Chaudhuri and Marchio have demonstrated that bakuchiol effectively reduces acne and is more effective when combined with salicylic acid.[23] Table 1.9 sets forth their findings presented as percent reduction in acne using the Global Acne Grading System.[60] Based on the results, formulations containing the combination of 1% bakuchiol and 2% salicylic acid showed a nearly 70% reduction in acne lesions and inflammation, as judged by the acne grading system. The next best results was attained with the 1% bakuchiol by itself, which reduced acne by a score of about 57%; whereas 2% salicylic acid only reduced acne by about 48%. As expected, practically no improvement in the reduction of acne was evident in the control group. None of the subjects observed or reported any adverse reaction using these formulated products. These results clearly show that bakuchiol is an effective ingredient, especially when combined with an exfoliating agent like salicylic acid, for the treatment of acne.

TABLE 1.9

Percent Reduction in Acne after Bakuchiol Treatment

Group #	Type of Lotions	Number of Volunteers	% Reduction in Acne after Treatment		
			2 weeks	4 weeks	6 weeks
1	1% bakuchiol	13[a]	30	42	57
2	2% salicylic acid	14[b]	21	34	48
3	1% bakuchiol +2% salicylic acid	14[a]	26	48	67
4	control	15	5	5	11

[a] Two dropped out due to protocol violation.

[b] One dropped out due to protocol violation.

Based on their findings, Chaudhuri and Marchio also concluded that bakuchiol is a multitasking product for mitigating acne-affected skin. It works by down-regulating 5α-reductase; inhibiting, if not killing, *P. acne* and other bacteria and fungus present in acne-affected skin; quenching radicals and non-radicals, especially inhibiting lipid peroxidation; reducing pro-inflammatory activity; and inhibiting matrix metalloprotease activity. Interestingly, these authors also reported that tazarotene-inducible gene 1 (TIG1) is significantly up-regulated by both bakuchiol and retinol (see Table 1.1), and the expression of TIG1 is found to be down-regulated in a variety of human cancers as well as acne, rosacea, and psoriasis. Thus, it is quite conceivable to assume that the up-regulation of TIG1 gene by bakuchiol may provide a solution to many skin problems in addition to acne.[23]

Skin Lightening and Even Toning

Photoaging is also associated with a dysregulation in melanin synthesis and distribution and with a general increase in the inflammatory status of the skin leading to the appearance of brown spots and an increase in skin redness. Recently, bakuchiol was shown to inhibit melanin production in a dose-dependent manner without showing strong cytotoxicty.[14] The results of that study, which included a comparison to arbutin, a known skin lightening agent, are summarized in Table 1.10. As noted, bakuchiol showed more than a 10-fold increase in activity as compared to arbutin.

These authors' findings also indicated that the addition of bakuchiol to the cells prior to stimulation with α-MSH markedly decreased the production of melanin in a dose-dependent manner. By applying the bakuchiol prior to α-MSH stimulation, the authors effectively showed that, at least in this regard, the effect is not due to tyrosinase inhibition: the primary mode of action of arbutin and other key skin whitening agents. Independently, Chaudhuri found that bakuchiol and retinol are very weak tyrosinase inhibitors. At 10 μg/mL level, bakuchiol and retinol have shown tyrosinase (mushroom) inhibitory activity of about 10% and 25%, respectively. EC_{50} could not be determined due to cytotoxicity at higher doses.

It has also been found that human skin exposed to UVB irradiation with a dose of 2 MED manifests a significant increase in the expression of Endothelin-1 (ET-1) and tyrosinase mRNA signals five days after irradiation.[61] In these studies, low levels of ET-1 secreted by keratinocytes in response to UVB radiation was shown to down-regulate E-cadherin in melanocytic cells. ET-1 is a potent down-regulator of E-cadherin in human melanocytes and also melanoma cells.[62] An independent and unpublished study by Chaudhuri has shown a sixfold up-regulation of CDH-1 gene coding for E-cadherin as compared to

TABLE 1.10

Effects of Bakuchiol on Melanin Production and Cell Viability in B16 Melanoma Cells

Compounds	Melanin/EC_{50} in μg/mL	Cell Viability/IC_{50} in μg/mL
Bakuchiol	1.8	5.9
Arbutin	24.0	>1000

Source: Adapted from Jamal S, Schneider RJ. *J Clin Invest* 2002;110:443–52.

a control in UV-B irradiated normal human keratinocytes treated with bakuchiol using 0.5 μg/mL. In light of the foregoing, it is quite tempting to propose that bakuchiol also reduces UV-induced hyperpigmentation by modulating E-cadherin. Additionally, in a small, open-label, pilot study by Shalita, it was found that 0.6% bakuchiol cream was effective and very well tolerated in reducing acne related post-inflammatory hyper-pigmentation. In light of the foregoing, it would seem that the combination of several skin lightening agents, targeting different pathways, may have additive or synergistic effects with bakuchiol at doses that may confer cost-effective and safe even toning as well as anti-aging effects.

Antimicrobial

Bakuchiol has shown bactericidal effects against *Streptococcus mutans*, *S. sanguis*, *S. salivarius, S sobrinus, Enterococcus faecalis, E. faecium, Lactobacillus acidophilus, L. casei, L. plantarum, Actinomyces viscosus,* and *Porphyromonas gingivalis,* with minimum inhibitory concentrations (MICs) ranging from 1 to 4 μg/mL and the sterilizing concentration (15 min exposure) ranging from 5 to 20 μg/mL.[27] In another study, an ether extract of *P. corylifolia* seed showed antimicrobial activity against various strains of bacteria. This study concluded that the antimicrobial activity was due to the presence of bakuchiol which, among other effects, inhibited the cell growth of *S. mutans* in a concentration dependent-manner and completely prevented growth at 20 μg/mL of bakuchiol.[63] Similarly, an *in vitro* screening of crude methanolic seed extract of *P. corylifolia* showed significant antimycobacterial activity against *Mycobacterium aurum* and *M. smegmatis* at a MIC of 62.5 μg/mL.[64] Recently, a new source of bakuchiol was found by bioassay-guided isolation from dried leaves of *Aerva sangulnolenta* Blume and shown to have good antibacterial activity against *S. mutans, A. viscosus, S. sanguis,* and moderate antifungal activity against *Malassezia furfur*.[15]

Chaudhuri has also demonstrated excellent antimicrobial activities of bakuchiol in an, as yet, unpublished work. Specifically, Chaudhuri conducted an evaluation to assess the minimum inhibitory concentration values (MIC in μg/mL) of bakuchiol against various organisms relevant to personal care applications in accordance with U.S. Pharmacopeia's Compendia Products procedure for Category 2 (USP 26–87, pp. 2022–2026). The results are given in Table 1.11. The data clearly shows that bakuchiol is an effective antimicrobial ingredient for use in personal care products. Additionally, a comparative study was done of the effectiveness of several commercial antimicrobial additives against *E. coli* and *S. aureus*: the results of that study are presented in Table 1.12. As indicated, bakuchiol is comparative with, if not a superior option to, current commercial antimicrobial additives.

TABLE 1.11

Minimum Inhibitory Concentration (MIC) Values of Bakuchiol Against Various Organisms

Organisms	MIC Value (μg/mL)
Bacteria	
E. coli	1.0
S. aureus	2.0
S. epidermidis	1.5
Streptococcus	4.0
Lactobacillus	3.0
P. gingivalis	1.0
P. acne	1.2
Pseudomonas aeruginosa	8.5
Fungi	
Aspergillus niger	0.8
Candida albican	1.5
P. ovale	25.8

TABLE 1.12

Comparative Inhibitory Activity of Bakuchiol vs. Leading Antimicrobial Ingredients

	MIC in μg/mL	MIC in μg/mL
Ingredients	*S. aureus*	*E. coli*
Bakuchiol	2.0	1.0
Chlorhexidine	0.5–1.0	1.0
Hexachlorophene	0.5	12.5
Cetrimide	4.0	16.0
Triclosan	0.1	5.0
Benzalkonium chloride	0.5	50.0

Other Targets

Protein Tyrosine Phosphatases (PTPs)

Phosphorylation and dephosphorylation of structural and regulatory proteins are major intracellular control mechanisms in eukaryotes. PTPs are a group of enzymes that remove phosphate groups from phosphorylated tyrosine residues on proteins.[33] Protein tyrosine (pTyr) phosphorylation is a common post-translational modification that can create novel recognition motifs for protein interactions and cellular localization, affect protein stability, and regulate enzyme activity. These enzymes are key regulatory components in signal transduction pathways (such as the MAP kinase pathway) and cell cycle control, and are important in the control of cell growth, proliferation, differentiation, and transformation. As a consequence, maintaining an appropriate level of protein tyrosine phosphorylation is essential for many cellular functions.

Bioassay-guided fractionation of the EtOAc-soluble extract of the seeds of *P. corylifolia* afforded two protein tyrosine phosphatase (PTP) 1B inhibitory compounds, psoralidin and bakuchiol, along with inactive corylin. Psoralidin and bakuchiol inhibited PTP1B activity in a dose-dependent manner, displaying IC_{50} values of 9.4 ± 0.5 μM and 20.8 ± 1.9 μM, respectively.[65] Thus, this is an area ripe for continued investigation.

DNA Polymerases

DNA polymerases are enzymes that are essential for DNA replication and are involved in a number of related cell processes, good and bad. DNA polymerase inhibitors, as their name suggests, are compounds that inhibit DNA polymerase activity. One key DNA polymerase inhibitor, resveratrol (Figure 1.2), was tested by Sun et al. and was found to have an inhibitory activity of 10 μM in an SV40 viral DNA replication assay.[66] More detailed structure–function analysis showed that resveratrol, whose structure has a 4-hydroxystyryl moiety in a trans conformation with respect to the m-hydroquinone, inhibits DNA polymerases α and δ (IC_{50} 3.3 and 5 μM, respectively) and, by comparison with structurally related resveratrol derivatives, demonstrated the absolute requirement of the 4-hydroxystyryl moiety for inhibition to occur.[67] Interestingly, both corylifolin and bakuchiol also possess the 4-hydroxystyryl moiety. Additionally, bioassay-directed purification of *P. corylifolia* ethanol extracts led to the identification of corylifolin and bakuchiol as DNA polymerase inhibitors.[66] Hence, inhibition of DNA synthesis provides yet another molecular mechanism for the chemopreventive activity of bakuchiol.

Tumor Suppressor p53

Anti-tumor activity of bakuchiol was investigated on the human lung adenocarcinoma A549 cell line. MTT assay revealed that the IC_{50} of bakuchiol at 72 h was 9.58 ± 1.12 μmol/L, much more effective than

that of resveratrol (33.02 ± 2.35 μmol/L). Bakuchiol has also been shown to reduce the mitochondrial membrane potential of cells in a concentration- and time-dependent manner. In fact, bakuchiol is shown to be more potent in many respects than resveratrol, producing/inducing a much higher level of apoptotic cells than resveratrol.[29] Additionally, p53 up-regulation, S phase arrest, caspase 9/3 activation, Bax up-regulation, and Bcl-2 down-regulation were observed in bakuchiol-treated A549 cells. These results suggest that S phase-related cell cycle regulation and, more importantly, reactive oxygen species-related apoptosis, might contribute to the anticancer properties of bakuchiol.

In another study, Russo et al.* showed that *P. glandulosa* extracts inhibited the growth of cancer cells after 48 h of treatment (IC_{50} of 10.5 μg/mL). The authors demonstrated that the extract induced apoptotic cell death, which they could attribute to the overall action of the meroterpenes present in the extract: the most active meroterpenes being bakuchiol, 12-hydroxy-iso-bakuchiol, 3-hydroxy-bakuchiol, and bakuchiol acetate. To a large extent, apoptotic cell death corresponded to a high level of DNA fragmentation, which, in turn, correlated to a significant increase in caspase-3 enzyme activity and Bax protein levels and a decrease in Bcl-2. This work supports the premise of the authors for the use of *P. glandulosa* as a potential source of anticancer agents, including, especially bakuchiol, for the treatment of melanoma.

Cellular tumor antigen p53, which is also known as phosphoprotein p53, tumor suppressor p53, and, simply p53, is a protein that, in humans, is encoded by the *TP53* gene. The p53 protein is crucial in multicellular organisms where it regulates the cell cycle and, thus, functions as a tumor suppressor, preventing cancer. As such, p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation. In its anti-cancer role, p53 works through several mechanisms: activating DNA repair proteins when DNA has sustained damage; arresting growth by holding the cell cycle at the $G2_1$/S regulation point on DNA damage recognition (if it holds the cell here for long enough, the DNA repair proteins will have time to fix the damage and the cell will be allowed to continue the cell cycle); and initiating apoptosis—programmed cell death—if DNA damage proves to be irreparable.[68] Thus, bakuchiol's up-regulation of p53, as noted above, adds further support to the use of this compound in preventing and/or treating cancer.

Signal Transducer and Activator of Transcription 3(STAT3)

Inhibiting interleukin-6 (IL-6) has been postulated as an effective therapy in the pathogenesis of several inflammatory diseases. Lee et al. have shown that bakuchiol has an inhibitory effect on IL-6-induced STAT3 promoter activity in Hep3B cells with an IC_{50} value of 4.57 ± 0.45.[69] In response to cytokines and growth factors, STAT family members are phosphorylated by receptor-associated kinases and then form homo- or heterodimers that translocate to the cell nucleus, where they act as transcription activators. STAT3 is essential for the differentiation of the TH17 helper T cells, which have been implicated in a variety of autoimmune diseases.[70]

Hypoxia Inducible Factor 1 (HIF-1)

A methanol extract of the seeds of *P. corylifolia* potently inhibited hypoxia inducible factor-1 (HIF-1) activation induced by hypoxia (100% inhibition at 20 μg/mL) in a HIF-1-mediated reporter gene assay.[71] Interestingly, bakuchiol is the only HIF-1 inhibitory agent (IC_{50} value 6.1 μM) found in this plant. In an effort to better understand the structural/performance relationship, the authors prepared few simple bakuchiol analogs and evaluated their HIF-1 inhibitory activities. Based on the results, the authors concluded that the phenolic hydroxyl group and the 12,13-double bond of bakuchiol play important roles in the biological activity of bakuchiol in HIF-1 inhibition.

HIF-1 is primarily involved in the sensing and adapting of cells to changes in the O_2 level, which is essential for their viability. A body of evidence indicates that oxygen deficiency clearly influences some major intracellular pathways such as those involved in cell proliferation, cell cycle progression, apoptosis, cell adhesion, and others.[71] HIF-1 is considered a central regulator of the adaptation response

* This work was presented by A Russo et al. at the 36 Congresso Nazionale Della Societa Italiana di Farmacologica held in Torino, 2013.

of cancer cells to hypoxia that makes it a therapeutic target in solid tumors. Hypoxia may induce changes in gene expression. Many genes involved in extracellular matrix remodeling are induced by hypoxic exposure. Matrix metalloproteases (MMPs) have also been implicated in metastatic progression, because MMPs can degrade all constituents of the basement membrane as well as structural components of the stroma.[72]

Conclusion

In summary, it is quite clear from the author's own work and the current literature that bakuchiol mimics and, in some cases, exceeds the activity of retinol towards various retinol targets and shows significant activity with respect to a number of non-retinol targets as well. Mechanistically, both the 4-hydroxystyryl and terpenic moieties of bakuchiol seem to be important, if not critical, with respect to the determination of its bioactive and physiological properties. Individual properties or effects, many of which are similar to retinol, may depend on the interplay between bakuchiol and very specific cellular targets that are upstream controllers of many cellular events. Overall, the complex and expansive biological action of bakuchiol and its capacity to modulate multiple different and distinct physiological pathways support the hypothesis of a mechanism involving multiple molecular targets. Thus, future studies on the properties of bakuchiol should evaluate the impact of bakuchiol on the maximal number of reported targets and their implications in topical as well as other modes of delivery.

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