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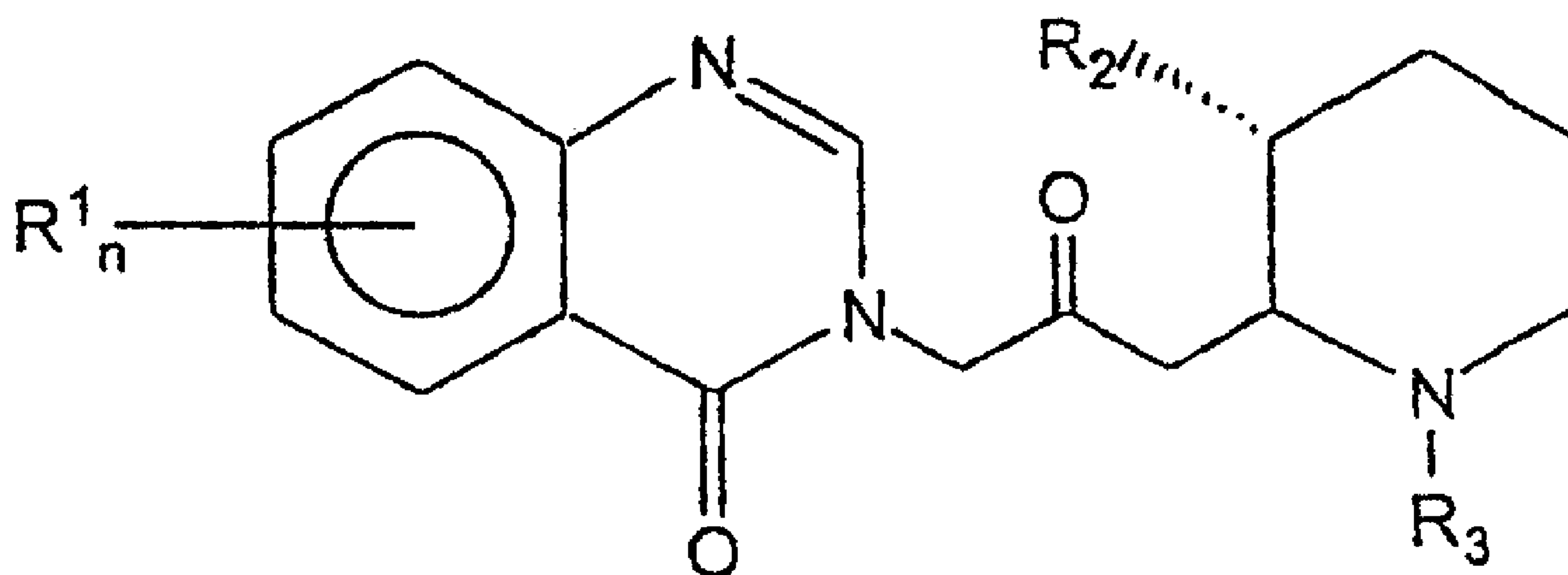
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(57) Abrégé/Abstract:

An effective treatment for skin disorders characterized by abnormal skin cell behavior, the treatment including a pharmaceutically effective amount of Halofuginone and pharmaceutically acceptable salts thereof. Skin disorders which can be treated include keloids, hypertrophic scars, psoriasis, acne, seborrhea and alopecia. Halofuginone can reduce or eliminate clinical symptoms of these disorders, as well as substantially prevent the formation of keloids and hypertrophic scars.

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(54) Title: TREATMENT OF SKIN DISORDERS (57) Abstract An effective treatment for skin disorders characterized by abnormal skin cell behavior, the treatment including a pharmaceutically effective amount of Halofuginone and pharmaceutically acceptable salts thereof. Skin disorders which can be treated include keloids, hypertrophic scars, psoriasis, acne, seborrhea and alopecia. Halofuginone can reduce or eliminate clinical symptoms of these disorders, as well as substantially prevent the formation of keloids and hypertrophic scars.		

TREATMENT OF SKIN DISORDERS

FIELD AND BACKGROUND OF THE INVENTION

5 The present invention relates to a method and a composition for the treatment of skin disorders and, more particularly, to a method and a composition for the treatment and prevention of psoriasis, hypertrophic scars and keloids.

Keloids are benign fibrotic tumors which are believed to arise from the reticular dermis. They are characterized by increased tissue fibrosis and collagen deposition [Friedman, D.W. *et al.*, *J. Surg. Res.*, Vol. 55, p. 214-222, 1993]. Keloids usually first appear when a patient is
10 between the ages of 10 and 30 years, and are often associated with trauma. They occur most commonly on the upper back, anterior chest, shoulders and ear lobes. Keloids are especially frequently seen in patients of African or Asian descent.

Hypertrophic scars are somewhat related to keloids, in that they are also characterized by increased tissue fibrosis and collagen deposition [Friedman, D.W. *et al.*, *J. Surg. Res.*, Vol. 55, p. 214-222, 1993]. Furthermore, hypertrophic scars are also most often seen in patients of
15 African and Asian descent [Rockwell, W.B. *et al.*, *Plastic and Recon. Surg.*, Vol. 84, p. 827-835, 1989]. Although there are certain differences between hypertrophic scars and keloids, such as a lower fibroblast density in keloids than in hypertrophic scars, a common mechanism is believed to underlie both conditions. Specifically, a genetically-determined aberration of the
20 metabolism of melanocyte-stimulating hormone (MSH) is believed to be responsible for both hypertrophic scars and keloids [Rockwell, W.B. *et al.*, *Plastic and Recon. Surg.*, Vol. 84, p. 827-835, 1989]. Thus, both hypertrophic scars and keloids are caused by genetically abnormal behavior of skin cells.

Keloids and hypertrophic scars are characterized histologically by a rich vasculature, a
25 high mesenchymal cell density, a thickened epidermis cell layer, and an abundance of collagen fibers. In hypertrophic scars, these fibers are loosely arrayed in a swirl-like pattern within bundles. In keloids, these fibers show even less organization, without any discrete bundles. By contrast, in normal skin these collagen fibers are arranged in distinct, clearly demarcated bundles.

30 The formation of both keloids and hypertrophic scars is marked by an initial infiltration of the traumatized tissue by fibroblasts, which is followed by the formation of a dense collagenous meshwork. Collagen production, as measured by prolyl hydroxylase activity, was found to be elevated in keloids, as compared to normal skin and normally healing wounds

[Cohen, K.I. *et al.*, *Surg. Forum*, Vol 22, p. 488, 1971]. Collagen synthesis was also found to be elevated in hypertrophic scars, but not to as great an extent [Rockwell, W.B. *et al.*, *Plastic and Recon. Surg.*, Vol. 84, p. 827-835, 1989]. The ratio of type I collagen to type III collagen was found to be significantly elevated in keloids but not hypertrophic scars, due to a specific increase in $\alpha 1(I)$ collagen gene expression, although type III collagen gene expression is also increased [Friedman, D.W. *et al.*, *J. Surg. Res.*, Vol. 55, p. 214-222, 1993; Rockwell, W.B. *et al.*, *Plastic and Recon. Surg.*, Vol. 84, p. 827-835, 1989]. Thus, clearly the deposition of collagen plays an important role in keloid and hypertrophic scar formation.

Similarly, psoriasis is also characterized by genetically-determined abnormal behavior of skin cells. Psoriasis is clinically marked by extensive scaling and a thickened epidermis [G.D. Weinstein and J.L. McCullough, *Cell Proliferation Kinetics*, p. 327-342]. These clinical manifestations are caused by hyperproliferation of epidermal cells. This hyperproliferation is also seen in non-psoriatic skin of psoriatic patients, indicating that the genetic defect is also present in apparently "normal" skin cells of psoriatic patients [G.D. Weinstein and J.L. McCullough, *Cell Proliferation Kinetics*, p. 327-342]. Although collagen also plays a role in the etiology of psoriasis, the abnormal hyperproliferation of epidermal cells is linked to the increased deposition of a number of extracellular matrix components, including collagen. Thus, clearly the inhibition of these extracellular matrix components could be an important factor in the inhibition of hyperproliferation by genetically abnormal psoriatic cells.

Keloids, hypertrophic scars and psoriasis thus have a number of characteristics in common. First, all three conditions are the result of a genetic defect in skin cells, which causes these cells to behave abnormally. Second, collagen plays a crucial, if varied, role in the development of all three conditions. However, abnormalities in collagen metabolism alone do not account for all of the abnormal behavior of the cells. Third, these skin disorders represent a significant cosmetic problem, particularly when present on the face, where they can be highly disfiguring and a source of considerable distress to the patient. Finally, these skin disorders can also cause patient discomfort, and depending upon the location of the affected area, can even have more severe consequences. For example, these disorders can cause pruritus and even pain. Individual keloids and hypertrophic scars can become so large that they are crippling [D.D. Datubo-Brown, *Brit. J. Plas. Surg.*, Vol 43, p. 70-77, 1990]. Furthermore, although keloids on the cornea are rare, such keloids can potentially result in blindness [D.D. Datubo-Brown, *Brit. J. Plas. Surg.*, Vol 43, p. 70-77, 1990]. Thus, keloids, hypertrophic scars and psoriasis have a range of consequences and effects, ranging from the relatively mild to highly

damaging effects.

Unfortunately, currently available treatments to inhibit the formation and growth of keloids and hypertrophic scars, and to treat psoriasis, are not completely successful. For example, surgery can be used to reduce the size or extent of the lesion, while physical pressure
5 can be used to reduce the size and extent of keloids and hypertrophic scars, as well as to prevent their initial formation [D.D. Datubo-Brown, *Brit. J. Plas. Surg.*, Vol 43, p. 70-77, 1990]. However, neither treatment can prevent the lesion from recurring, and surgery especially carries a risk of increased morbidity.

Other forms of treatment include the administration of corticosteroids. For example,
10 triamcinolone appears to reduce the size of keloids and hypertrophic scars by increasing the rate of collagen degradation [Rockwell, W.B. *et al.*, *Plastic and Recon. Surg.*, Vol. 84, p. 827-835, 1989]. However, the side effects of such medications are potentially dangerous and are not universally successful. Other treatments, such as radiation, also showed variable effectiveness and are associated with other potential side effects [Rockwell, W.B. *et al.*, *Plastic and Recon.*
15 *Surg.*, Vol. 84, p. 827-835, 1989]. Thus, clearly improved treatments for keloids, psoriasis and hypertrophic scars are required.

As noted above, collagen synthesis and deposition plays an important role in keloid and hypertrophic scar formation, as well as in the cell hyperproliferation associated with psoriasis. The synthesis of collagen is also involved in a number of other pathological conditions,
20 particularly those associated with primary or secondary fibrosis. The crucial role of collagen in fibrosis has prompted attempts to develop drugs that inhibit the accumulation of collagen [K.I. Kivirikko, *Annals of Medicine*, Vol. 25, pp. 113-126 (1993)].

Such drugs can act by modulating the synthesis of the procollagen polypeptide chains, or by inhibiting specific post-translational events, which will lead either to reduced formation of
25 extra-cellular collagen fibers or to an accumulation of fibers with altered properties. Unfortunately, only a few inhibitors of collagen synthesis and deposition are available, despite the importance of this protein in sustaining tissue integrity and its involvement in various disorders. Furthermore, many available inhibitors lack specificity for the collagen metabolic pathway. Thus, many currently available drugs have deleterious side effects.

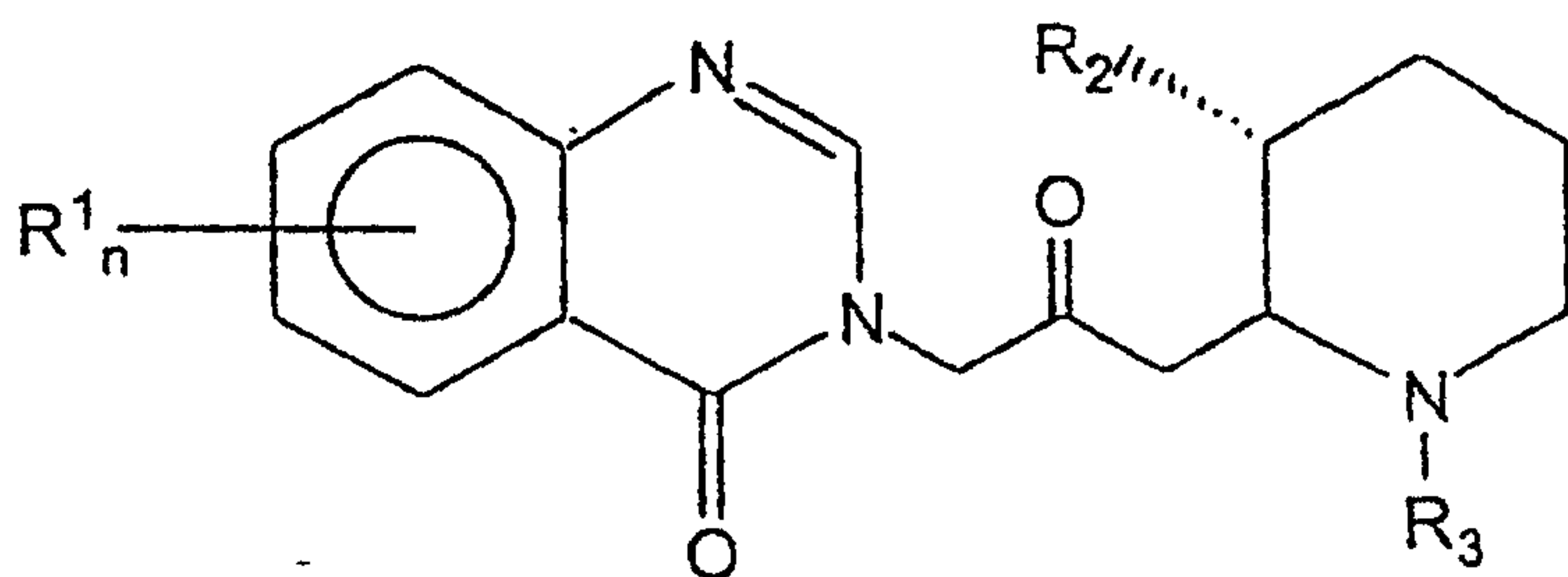
30 For example, cytotoxic drugs have been used in an attempt to slow the proliferation of collagen-producing fibroblasts [J.A. Casas, *et al.*, *Ann. Rhem. Dis.*, Vol. 46, p. 763 (1987)], such as colchicine, which slows collagen secretion into the extracellular matrix [D. Kershenobich, *et al.*, *N. Engl. J. Med.*, Vol. 318, p. 1709 (1988)]. Other drugs act as inhibitors of key collagen

metabolism enzymes [K. Karvonen, *et al.*, *J. Biol Chem.*, Vol. 265, p. 8414 (1990); C.J. Cunliffe, *et al.*, *J. Med. Chem.*, Vol. 35, p.2652 (1992)]. However, none of these inhibitors have specific effects for the metabolism and deposition of specific types of collagen. Also, these drugs may interfere with the biosynthesis of other vital collagenous molecules, such as Clq in the classical complement pathway, acetylcholine esterase of the neuro-muscular junction endplate, conglutinin and pulmonary surfactant apoprotein. Such interference and lack of specificity could have potentially serious adverse effects.

Other drugs which can inhibit collagen synthesis, such as nifedipine and phenytoin, inhibit synthesis of other proteins as well, thereby non-specifically blocking the collagen biosynthetic pathway [T. Salo, *et al.*, *J. Oral Pathol. Med.*, Vol. 19, p. 404 (1990)]. Again, the lack of specificity significantly reduces the clinical use of these drugs, because the non-specific inhibition of protein synthesis can result in adverse side-effects when the drug is administered to the patient.

Indeed, clinically available anti-fibrotic drugs, including the collagen cross-linking inhibitors such as β -amino-propionitrile, are also non-specific. Unfortunately, the lack of specificity of these collagen cross-linking inhibitors ultimately results in severe side effects after prolonged use. These side effects include lathritic syndrome, as well as disrupted elastogenesis. The latter side effect is a result of the disruption of cross-linking of elastin, another fibrous connective tissue protein. In addition, the collagen cross-linking inhibitory effect of these drugs is secondary, so that collagen must first be overproduced before degradation by collagenase. Thus, a type-specific inhibitor of the synthesis of collagen itself is clearly required.

Such a type-specific collagen synthesis inhibitor is disclosed in U.S. Patent No. 5,449,678 for the treatment of certain fibrotic conditions such as scleroderma and Graft Versus Host Disease. Both of these conditions are associated with excessive collagen deposition, which can be inhibited by Halofuginone. This specific inhibitor is a composition with a pharmaceutically effective amount of a pharmaceutically active compound of a formula:



wherein: $n=1$ or 2

R₁ is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R₂ is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R₃ is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl. Of this group
5 of compounds, Halofuginone has been found to be particularly effective for such treatment.

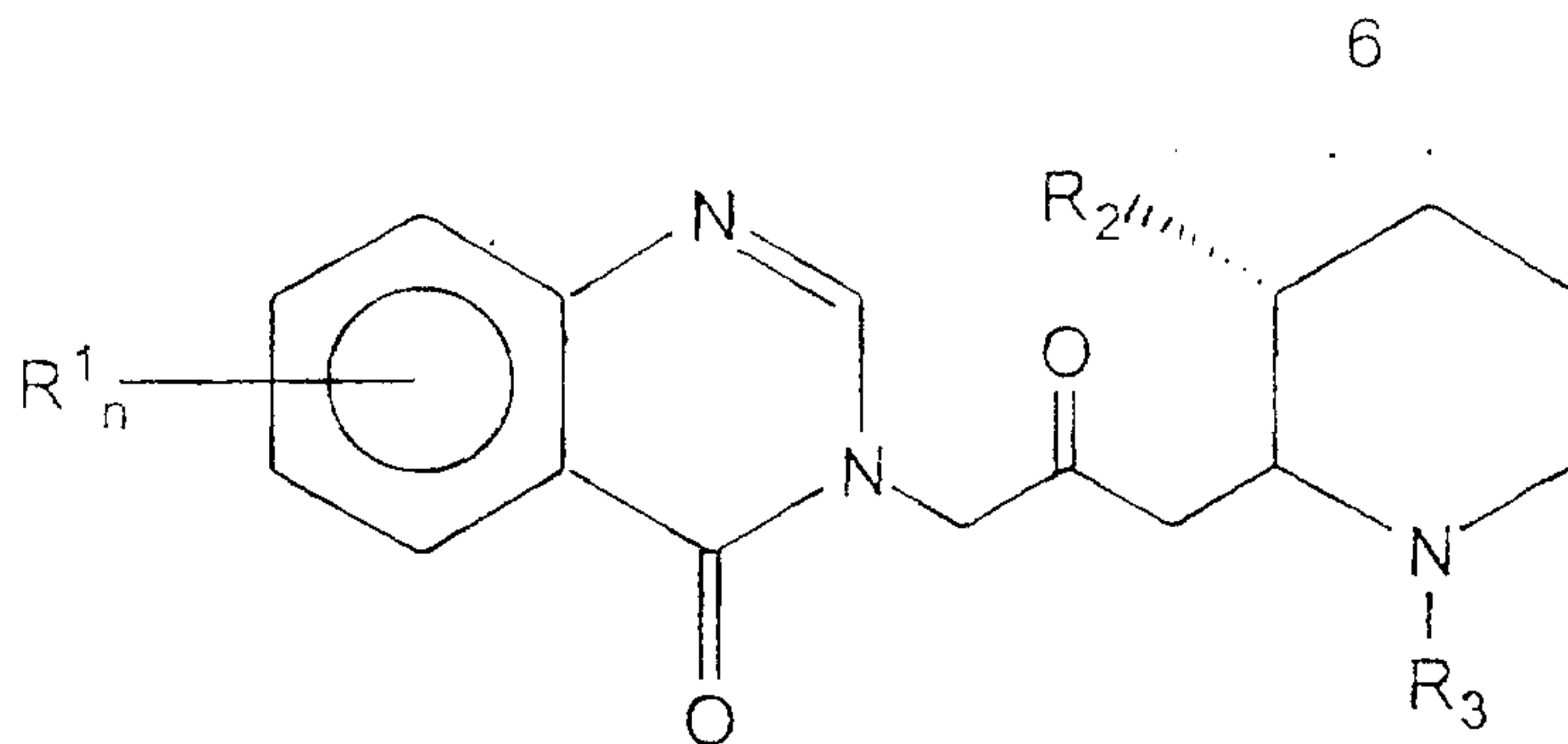
PCT Patent Application No. 96/06616 further discloses that these compounds are able to effectively treat restenosis by preventing the proliferation of vascular smooth muscle cells. Restenosis is characterized by smooth muscle cell proliferation and extracellular matrix accumulation within the lumen of affected blood vessels in response to a vascular injury [Choi
10 *et al.*, *Arch. Surg.*, Vol. 130, p. 257-261 (1995)]. One hallmark of such smooth muscle cell proliferation is a phenotypic alteration, from the normal contractile phenotype to a synthetic one. Type I collagen has been shown to support such a phenotypic alteration, which can be blocked by Halofuginone [Choi *et al.*, *Arch. Surg.*, Vol. 130, p. 257-261 (1995); PCT Patent Application No. 96/06616]. Thus, Halofuginone can prevent such abnormal redifferentiation
15 of smooth muscle cells after vascular injury by blocking the synthesis of type I collagen.

However, although the synthesis and deposition of type I collagen has been shown to play an important role in the pathogenesis of psoriasis, keloids and hypertrophic scars, many other factors are also important in the pathogenesis of these skin disorders. Indeed, current treatments for keloids and hypertrophic scars, such as surgical intervention, do not specifically
20 alter the metabolism of type I collagen. Thus, even the administration of a known specific type I collagen synthesis inhibitor may not alter the pathogenesis of these skin disorders and thereby alleviate their associated clinical manifestations without causing adverse side effects.

There is thus a widely recognized unmet medical need for a medicament which specifically prevents the formation of keloids and hypertrophic scars, which specifically
25 prevents the pathogenesis of psoriasis, and which specifically reduces or alleviates psoriasis and already formed keloids and hypertrophic scars, without causing adverse side effects.

SUMMARY OF THE INVENTION

According to the present invention, there is provided a composition for treating a skin
30 disorder characterized by substantially abnormal cell behavior, comprising a pharmaceutically effective amount of a compound in combination with a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



wherein: $n=1$ or 2

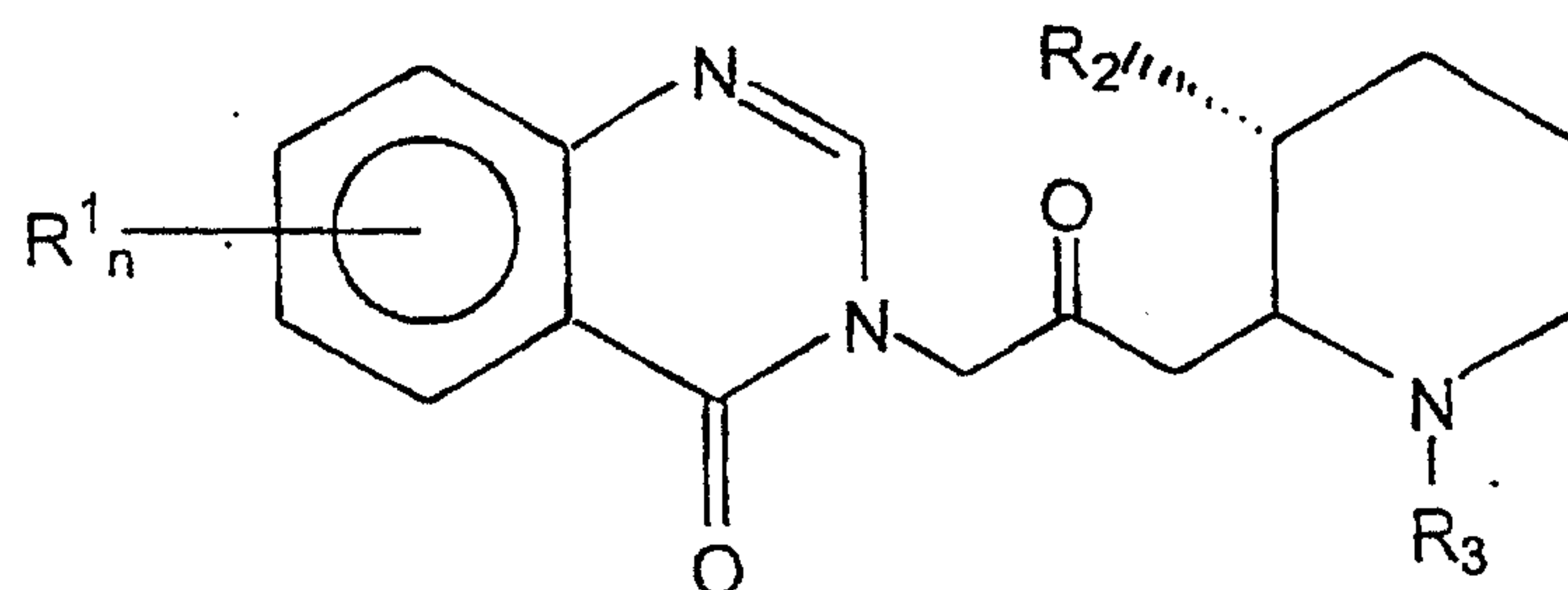
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

5 R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy.

Preferably, the compound is Halofuginone. Also preferably, the skin disorder is selected from the group consisting of psoriasis, keloid, hypertrophic scar, acne, seborrhea and alopecia. More preferably, the skin disorder is selected from the group consisting of psoriasis, keloid and
10 hypertrophic scar.

According to another embodiment of the present invention, there is provided a method for manufacturing a medicament for treating a skin disorder characterized by substantially abnormal cell behavior, comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, the compound being a member of a group
15 having a formula:



wherein: $n=1$ or 2

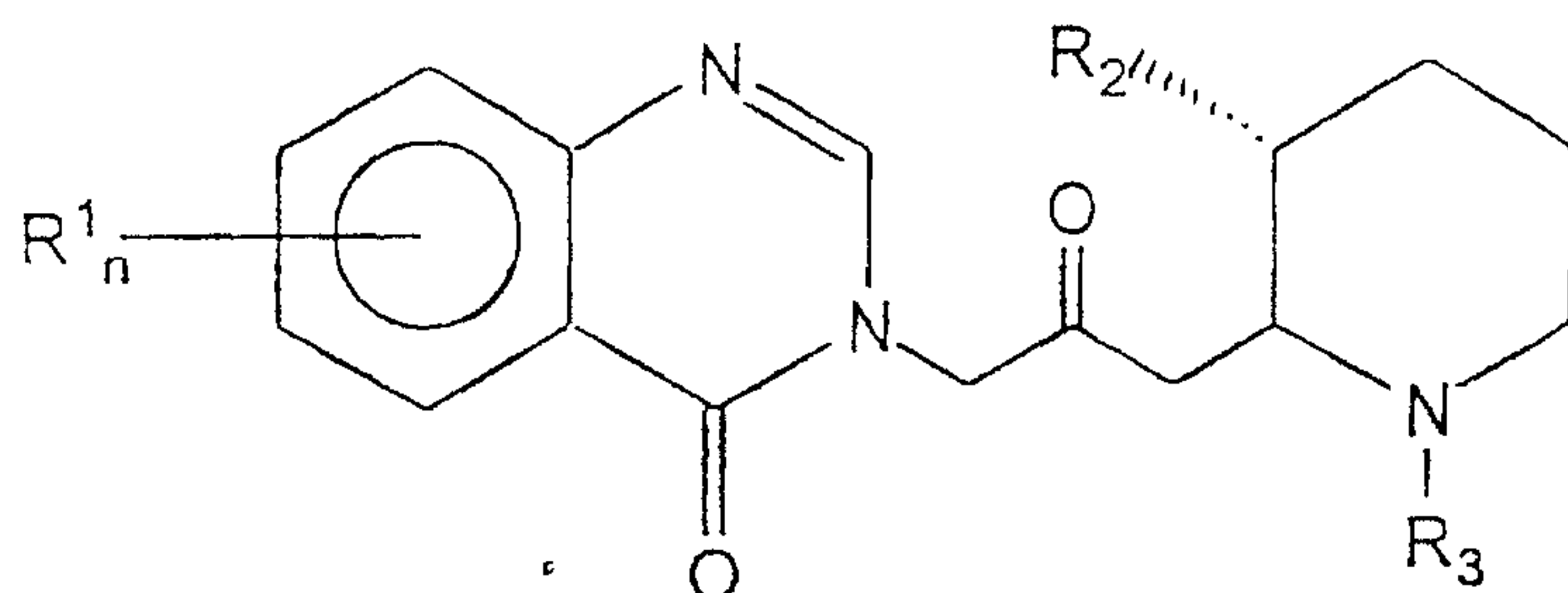
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

20 R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl.

Preferably, the compound is Halofuginone. Also preferably, the skin disorder is selected from the group consisting of psoriasis, keloid, hypertrophic scar, acne, seborrhea and alopecia. More preferably, the skin disorder is selected from the group consisting of psoriasis, keloid and
25 hypertrophic scar.

According to yet another embodiment of the present invention, there is provided a method of manufacturing a medicament for inhibiting formation of a keloid-like growth, the growth being caused by a performance of a surgical procedure, the method comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, the compound being a member of a group having a formula:



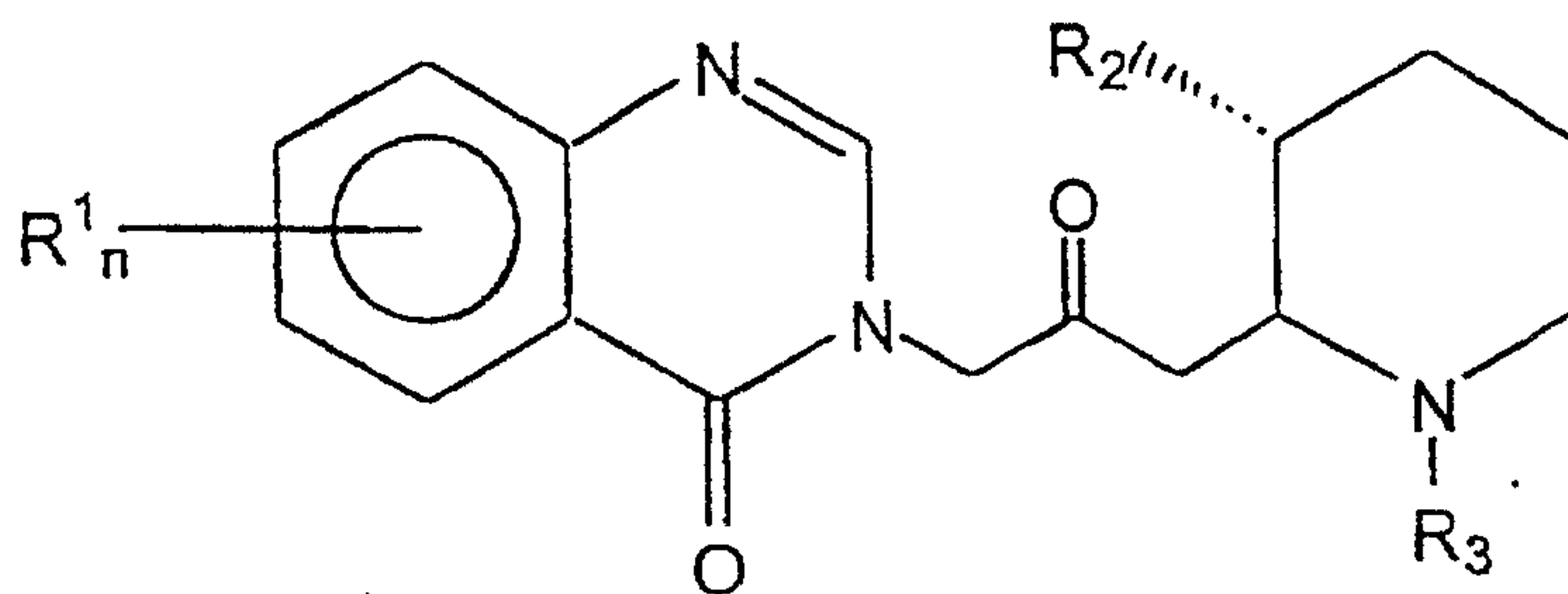
wherein: $n=1$ or 2

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

10 R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl.

According to still another embodiment of the present invention, there is provided a composition for inhibiting formation of a keloid-like growth in a subject, the growth being caused by a performance of a surgical procedure on the subject, the composition comprising a pharmaceutically effective amount of a compound having a formula:



wherein: $n=1$ or 2

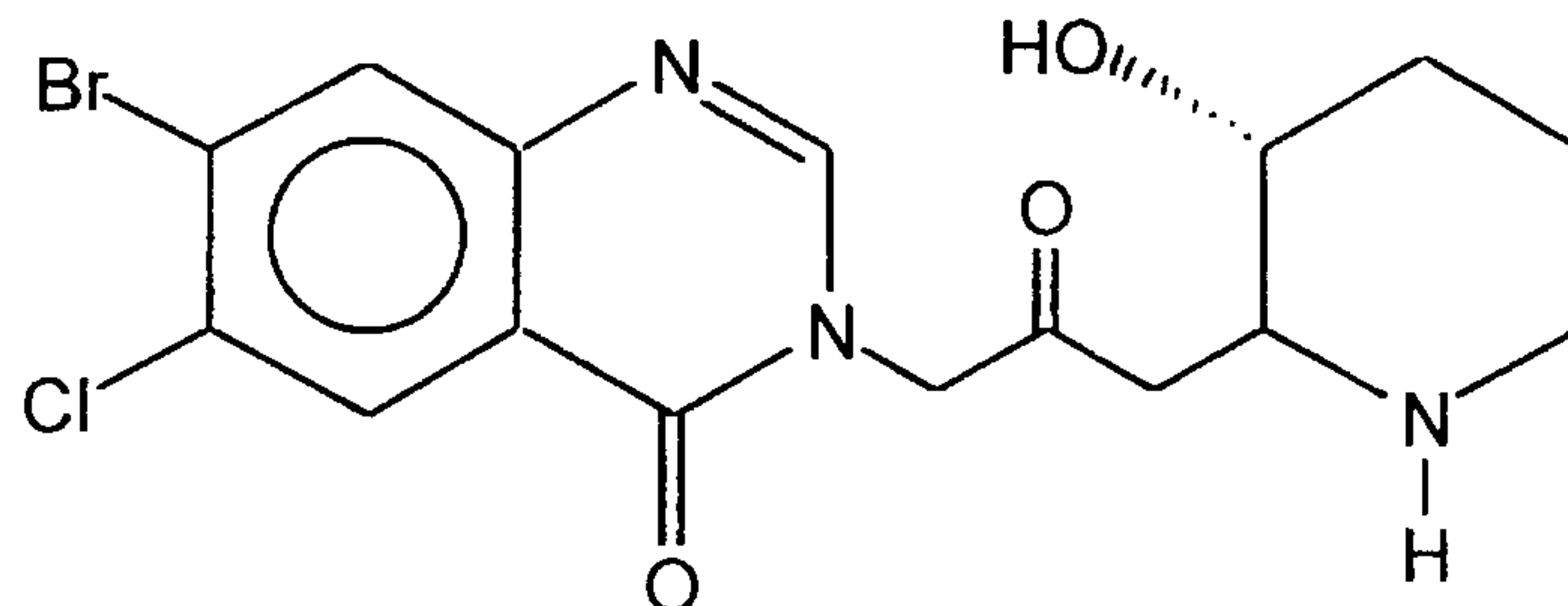
R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

20 R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl.

Preferably, the composition is administered to the subject substantially before the performance of the surgical procedure. Alternatively and preferably, the composition is administered to the subject substantially after the performance of the surgical procedure.

In each of the embodiments above, preferably the compound is Halofuginone. Hereinafter, the term "Halofuginone" is defined as a compound having a formula:



5 and pharmaceutically acceptable salts thereof. The composition preferably includes a pharmaceutically acceptable carrier for the compound.

Preferably, all of the compounds referred to hereinabove can be either the compound itself as described by the formula, and/or pharmaceutically acceptable salts thereof.

Further preferably, the skin disorder is selected from the group consisting of psoriasis,
 10 keloid, hypertrophic scar, acne, seborrhea and alopecia. Most preferably, the skin disorder is selected from the group consisting of psoriasis, keloid and hypertrophic scar. Hereinafter, the term "keloid-like growth" includes keloid and hypertrophic scar.

BRIEF DESCRIPTION OF THE DRAWINGS

15 The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIGS. 1A and 1B show collagen synthesis in keloid-derived tissue;

FIG. 2 illustrates the inhibitory effect of Halofuginone on collagen synthesis in keloid-derived tissue;

20 FIG. 3 shows Halofuginone inhibition of sulfate incorporation into ECM of cultured endothelial cells;

FIGS. 4A-4D show inhibition of incorporation of sulfate, proline, lysine and glycine into ECM of bovine corneal endothelial cells by Halofuginone; and

25 FIGS. 5A-5D show the inhibition of sulfate and glycine incorporation into rat mesengial cell ECM by Halofuginone.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unexpectedly, Halofuginone has been found to be an effective inhibitor of collagen synthesis by keloid-derived cells. Such an effect was not predicted by the prior art for the

following reasons. First, the prior art did not teach the treatment of keloids or hypertrophic scars with Halofuginone. Second, keloids and hypertrophic scars arise from genetically abnormal skin cells. Therefore, the behavior of these cells in response to Halofuginone cannot be predicted from the response of normal skin cells to Halofuginone. Third, keloids and hypertrophic scars are both characterized by the abnormal organization of collagen, as well as by over-synthesis of collagen. The prior art did not teach that Halofuginone would have any effect on pathological processes characterized by dysfunctional collagen fibril organization. Thus, the finding that Halofuginone is an effective inhibitor of keloid-related pathological processes is both novel and non-obvious.

Such a finding has implications for the treatment of other skin conditions as well, particularly psoriasis. As noted above, psoriasis is characterized by hyperplasia of the skin which is enabled by the deposition of excess extracellular matrix components (ECM), including collagen. Furthermore, psoriasis is also the result of a pathogenic process caused by genetically abnormal cells. Therefore, the use of Halofuginone to treat psoriasis is novel and non-obvious for the following reasons. First, the prior art did not teach the treatment of psoriasis with Halofuginone. Second, the inhibitory effect of Halofuginone on ECM formation was also not suggested nor was it taught by the prior art, yet as detailed in the examples below, Halofuginone completely inhibits deposition of ECM components. Third, as noted above, Halofuginone can alter the behavior of genetically abnormal skin cells, an effect which was not taught by the prior art. Thus, the treatment of psoriasis by Halofuginone is both novel and non-obvious.

Other examples of skin disorders which could be amenable to treatment with Halofuginone include acne, seborrhea and alopecia. These conditions all reflect abnormal skin cell environments. For example, acne is a disorder characterized by excess oil production by the skin, leading to bacterial infection and scar formation if untreated. However, excess oil production is promoted by the influence of hormones on skin cells, which is one reason adolescents tend to be most affected. Such hormones cause an abnormal environment for the skin cell, so that the cell in turn behaves abnormally. As noted above, Halofuginone has been shown to be effective in the control and inhibition of abnormal skin cell behaviors. Thus, Halofuginone is also a treatment for disorders, such as acne, characterized by such abnormal skin cell behavior.

Halofuginone can therefore be used to both prevent the clinical manifestations of skin disorders such as keloids, hypertrophic scars, psoriasis and acne, and to alleviate these

disorders once they have arisen. For example, as detailed below, Halofuginone has been shown to be effective as a pretreatment, before surgery, for another surgically-induced pathological process, the formation of adhesions. Thus, Halofuginone is effective as a pretreatment before the appearance of clinical symptoms, as well as being able to alleviate or substantially eliminate such symptoms after they appear.

The present invention may be more readily understood with reference to the following illustrative examples and figures. It should be noted that although reference is made exclusively to Halofuginone, it is believed that the other quinazolinone derivatives described and claimed in U.S. Patent No. 3,320,124, have similar properties.

Example 1

Collagen Synthesis in Keloid-Derived Tissue

The presence of large amounts of collagen protein, as well as of the expression of the collagen $\alpha 1(I)$ gene, were demonstrated in keloid-derived tissue. The results are shown in Figures 1A and 1B. The experiment was conducted as follows.

A keloid was removed from the ear lobe of a 21 year-old male. The keloid had arisen in response to tissue trauma caused by the piercing of the ear for insertion of an earring. The removed keloid tissue was sectioned so that histological studies could be performed. Briefly, the tissue samples were collected into phosphate-buffered saline (PBS) and fixed overnight in 4% paraformaldehyde in PBS at 4°C. Serial 5 μ m sections were prepared after the samples had been dehydrated in graded ethanol solutions, cleared in chloroform and embedded in Paraplast. Differential staining of collagenous and non-collagenous proteins was performed with 0.1% Sirius red and 0.1% fast green as a counter-stain in picric acid. This procedure stains collagen red [Gascon-Barre, M., *et al.*, *J. Histochem. Cytochem.*, Vol 37, p. 377-381, 1989]. The results are shown in Figure 1A.

For hybridization with the genetic probe, the sections were deparafinized in xylene, rehydrated through a graded series of ethanol solutions, rinsed in distilled water for 5 minutes and then incubated in 2X SSC at 70°C for 30 minutes. The sections were then rinsed in distilled water and treated with pronase, 0.125 mg/ml in 50 mM Tris-HCl, 5 mM EDTA, pH 7.5, for 10 minutes. After digestion, the slides were rinsed with distilled water, post-fixed in 10% formalin in PBS and blocked in 0.2% glycine. After blocking, the slides were rinsed in distilled water, rapidly dehydrated through graded ethanol solutions and air-dried for several

hours. The sections were then hybridized with a genetic probe.

Before hybridization, the genetic probe was prepared by cutting out the 1600 bp rat collagen $\alpha 1(I)$ insert from the original plasmid, pUC18. The 1600 bp insert was then inserted into the pSafyre plasmid. The sections were then hybridized with this probe after
5 digoxigenin-labelling. Alkaline phosphatase activity was detected in the sections as previously described [Knopov, V., *et al.*, *Bone*, Vol 16, p. 329S-334S, 1995]. The results are shown in Figure 1B.

Figure 1A shows a section of tissue taken from the keloid and stained with Sirius red. Most of the keloid tissue was stained with Sirius red except the epidermis, indicating the
10 presence of high concentrations of collagen within the tissue. Figure 1B shows the presence of cells, probably fibroblasts, which express high levels of the collagen $\alpha 1(I)$ gene. Thus, clearly the keloid tissue was actively synthesizing collagen and expressing the type I collagen gene.

15

Example 2

Inhibitory Effect of Halofuginone on Collagen Synthesis in Keloid-Derived Tissue

Halofuginone was shown to specifically inhibit collagen synthesis in keloid-derived tissue. The results are shown in Figure 2. The experiment was conducted as follows.

20 The keloid was removed as described in Example 1 above. The keloid-derived cells were incubated with and without Halofuginone for 24 hours in 0.5 ml glutamine-free DMEM containing 5% FCS (Fetal Calf Serum), ascorbic acid (50 $\mu\text{g/ml}$), B-aminopropionitrile (50 $\mu\text{g/ml}$) and 2 μCi of [^3H]proline. At the end of incubation, the medium was decanted and the cells were incubated with or without collagenase for 18 hours, followed by precipitation of
25 proteins by the addition of TCA (trichloroacetic acid). The amount of radiolabelled collagen was estimated as the difference between total labelled-proline containing proteins and those left after collagenase digestion [Granot, I. *et al.*, *Mol. Cell Endocrinol.*, Vol. 80, p. 1-9, 1991].

The ratio of collagenase digestible to non-collagenase digestible proteins was found to be higher in the keloid-derived cells than the usual values for normal skin cells. However,
30 Figure 2 shows that Halofuginone inhibited the production of collagen, but not of non-collagenase digestible proteins. Thus, Halofuginone specifically inhibited collagen synthesis in keloid-derived cells, and not total protein synthesis.

Example 3Halofuginone Inhibition of Sulfate Incorporation into
ECM of Cultured Endothelial Cells

As noted above, skin cell hyperproliferation is enabled by the deposition of ECM components. The following examples illustrate the ability of Halofuginone to inhibit such deposition of ECM components, further supporting the use of Halofuginone as a treatment for psoriasis. These examples illustrate the unexpected finding that Halofuginone completely abolishes deposition of all ECM components, and not just collagen, thereby preventing cell proliferation which is enabled by the formation of ECM.

Cultures of bovine corneal endothelial cells were established from steer eyes and maintained as previously described [D. Gospodarowicz, *et al.*, *Exp. Eye Res.*, No. 25, pp. 75-89 (1977)]. Cells were cultured at 37°C in 10% CO₂ humidified incubators and the experiments were performed with early (3-8) cell passages.

For preparation of sulfate-labelled ECM (extra-cellular matrix), corneal endothelial cells were seeded into 4-well plates at a confluent density forming, within 4-6 hours, a contact-inhibited cell monolayer composed of closely apposed, and growth arrested cells. Under these conditions, the cells remained viable and retained their normal monolayer configuration and morphological appearance up to a concentration of 2 µg/ml halofuginone. Na₂[³⁵S]O₄ (540-590 mCi/mmol) was added (40 µCi/ml) one and five days after seeding and the cultures were incubated without changing the medium. At various intervals after seeding, the subendothelial ECM was exposed by dissolving (5 min., room temperature) the cell layer with PBS containing 0.5% Triton X-100 and 20 mM NH₄OH, followed by four washes in PBS [I. Vlodavsky, *et al.*, *Cancer Res.*, Vol. 43, pp. 2704-2711 (1983); I. Vlodavsky, *et al.*, *Proc. Natl. Acad. Sci. USA*, Vol. 84, pp. 2292-2296 (1987)]. To determine the total amount of sulfate labeled material, the ECM was digested with trypsin (25 µg/ml, 24 h, 37°C) and the solubilized material counted in a β-counter.

Figure 3 shows the almost complete inhibition of sulfate incorporation by 1 µg/ml Halofuginone, while 50% inhibition was obtained in the presence of 0.2 µg/ml of the drug.

Example 4Inhibition of Incorporation of Sulfate, Proline, Lysine and Glycine
into ECM of Bovine Corneal Endothelial Cells

Corneal endothelial cells were seeded at a confluent density and grown as described in
5 Example 3 above. The cells were cultured with or without Halofuginone in the presence of
either $\text{Na}_2^{35}\text{SO}_4$ (Figure 4A), ^3H -proline (Figure 4B), ^{14}C -lysine (Figure 4C) or ^{14}C -glycine
(Figure 4D). Eight days after seeding, the cell layer was dissolved substantially as described
in Example 3 above. The underlying ECM was then either trypsinized to determine the effect
of Halofuginone on incorporation of labeled material into total protein, substantially as
10 described in Example 3 above, or subjected to sequential digestions with collagenase and
trypsin to evaluate the effect of Halofuginone on both collagenase-digestible proteins (CDP)
and non-collagenase digestible proteins (NCDP).

As Figures 4A-4D show, Halofuginone inhibited the incorporation of sulfate, proline,
lysine and glycine into both CDP and NCDP, reflecting a profound inhibition of matrix
15 deposition. The inhibitory effect of Halofuginone on deposition of ECM components other
than collagen is most likely due to the involvement of collagen in the assembly of other
constituents into the supramolecular structure of the ECM. Alternatively, Halofuginone may
affect the synthesis of ECM components other than collagen, possibly through a common
transcription factor or cytokine such as $\text{TGF}\beta$, which affects the synthesis and deposition of
20 several ECM components.

Example 5Inhibition of Sulfate and Glycine Incorporation into
Rat Mesengial Cell ECM

25 Rat mesengial cells were grown to confluency, 24 hours after seeding. The cells were
then cultured with or without Halofuginone in the presence of either $\text{Na}_2^{35}\text{SO}_4$ (Figures 5A
and 5B) or ^{14}C -glycine (Figures 5C and 5D). Eight days after seeding, the cell layer was
dissolved to expose the underlying ECM, washed and digested with collagenase to determine
the effect of Halofuginone on CDP proteins, as shown in Figures 5A and 5C. The remaining
30 material was digested with trypsin and subjected to β -scintillation counting to determine the
effect of Halofuginone on NCDP proteins, as shown in Figures 5B and 5D.

About 30% inhibition of sulfate incorporation was seen for CDP proteins, while about
70% inhibition was seen for NCDP proteins in the presence of 200 ng/ml Halofuginone.

Halofuginone did not inhibit ECM deposition by preventing cell proliferation since the drug was added to highly confluent, non-dividing cells. Since inorganic sulfate is incorporated primarily into sulfated glycosaminoglycans and not into collagen, Halofuginone may interfere with the assembly of other ECM macromolecules, such as heparin sulfate proteoglycans by inhibiting type I collagen synthesis. These other ECM macromolecules are known to specifically interact with collagen to form ECM, so that the lack of collagen might thus prevent the assembly of these macromolecules.

About 80% inhibition of glycine incorporation was seen for both CDP and NCDP proteins in the presence of 50 ng/ml Halofuginone. The inhibitory effect of Halofuginone on deposition of collagenase-digestible ECM proteins was more pronounced with glycine-labeled than with sulfate-labeled matrix since unlike glycine, sulfate is incorporated primarily into glucosaminoglycans which are not degraded by collagenase. A profound inhibition of ECM deposition was supported by a microscopic examination of the denuded culture dishes, revealing a thin or non-existent layer of ECM produced in the presence of Halofuginone.

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Example 6

Halofuginone as a Pretreatment

As noted above, Halofuginone has unexpectedly been shown to be effective as a pretreatment for the prevention of a surgically-induced pathological process, the formation of adhesions. Such an effect also has implications for the prevention of surgically-induced keloids and hypertrophic scars. The results of pretreatment with Halofuginone are given in Table 1 below.

The experimental method was as follows. Halofuginone was administered in the diet of two groups of rats at a concentration of 5 mg/kg dry feed for 4 days before surgery, as a pretreatment. Two other groups of rats were fed a normal diet and served as control groups. One of the groups of rats fed Halofuginone and one control group then underwent surgery, which was performed as follows. First, the abdomen of the rats was shaved and prepared with iodine and alcohol. The abdominal cavity was entered through a mid-line incision. The small intestine was scraped from the duodenum down to from about 9 to about 10 cm from the cecum, until capillary bleeding occurred. To avoid drying, Hartman's solution at about 37 °C was occasionally dripped on the intestine. After replacement of the intestine into the abdominal cavity, the abdomen was closed in two layers with continuous 00 chromic catgut suture. This method has been previously demonstrated to cause abdominal adhesions

[Rivkind, A.I. *et al.*, *Eur. Surg. Res.*, Vol 17, p. 254-258, 1985].

In those rats receiving the drug, Halofuginone treatment was continued for 21 days following surgery. At the end of 21 days, the rats were weighed and the number and severity of adhesions were determined according to a double-blind procedure, in which adhesions were
5 classified according to the following grading: 0=no adhesions; I=a thin, filmy, easily separated adhesion; II= several thin adhesions; III= a thick, broad adhesion and IV= several thick adhesions. Clearly, 0 is the least severe grade and IV is the most severe grade.

Table 1 shows the results of Halofuginone treatment on the number and severity of adhesions in rats which underwent surgery. The abbreviation "+Op, -H" refers to rats which
10 underwent surgery, without Halofuginone treatment. The abbreviation "+Op, +H" refers to rats which underwent surgery with Halofuginone treatment. The abbreviation "-Op, -H" refers to rats which did not undergo surgery and did not receive Halofuginone. The abbreviation "-Op, +H" refers to rats which did not undergo surgery but did receive Halofuginone. Halofuginone was administered i.p. (25 µg/kg body weight) and in the diet (5 mg/kg diet or
15 10 mg/kg diet). "Wt. gain (g)" refers to the average weight gain in grams as means $\pm$ SE. Means without a common superscript within a column differ significantly according to Duncan's Multiple Range Test.

When administered orally, Halofuginone was given in the diet for 4 days before surgery, as a pretreatment. Two other groups of rats were fed a normal diet and served as
20 control groups. One of the groups of rats fed Halofuginone and one control group then underwent surgery as described above. Halofuginone treatment was continued for 21 days following surgery, after which the rats were sacrificed and the number and severity of adhesions were determined, and body weight was measured.

Clearly, no adhesions were found in control rats which did not undergo surgery. Both
25 the number and severity of adhesions were sharply reduced in Halofuginone-treated rats as compared to non-treated rats, almost to the level seen in rats which did not undergo surgery. Most of the adhesions were located between loops of the small bowels and at least one adhesion was present between the small bowel and the omentum. A small reduction in weight gain was observed in nearly all rats which underwent surgery, although this reduction was
30 more pronounced in Halofuginone-treated rats.

Thus, clearly Halofuginone administered in the diet, similar to the administration to chickens as a coccidostat, was able to inhibit post-surgical adhesion formation in rats. Furthermore, weight gain by all of the different groups of rats was substantially similar,

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showing that the effect of Halofuginone was specific and did not result in any general reduction in overall well-being of the rats.

Table 1. Effect of Halofuginone on Abdominal Adhesions

Treatment	IP (25 µg/kg)			Dietary (5 mg/kg)			Dietary (10 mg/kg)		
	n	Wt. gain (g)	Adhesion score	n	Wt. gain (g)	Adhesion score	n	Wt. gain (g)	Adhesion score
-Op, -H	5	51±6.7 ^a	0	5	90±5.5 ^a	0	10	91±5.4 ^a	0
-Op, +H	-	-	-	4	95±4.8 ^a	0	10	83±3.4 ^a	0
+Op, -H	5	29±3.4 ^b	25	5	92±3.2 ^a	13	10	88±4.5 ^a	36
+Op, +H	5	11±7.1 ^c	5	6	84±3.7 ^a	6	10	71±4.8 ^a	13

Example 7Suitable Formulations for
Administration of Halofuginone

Halofuginone can be administered to a subject in a number of ways, which are well
5 known in the art. Hereinafter, the term "subject" refers to the human or lower animal to whom
Halofuginone was administered. For example, administration may be done topically (including
ophthalmically, vaginally, rectally, intranasally), orally, or parenterally, for example by
intravenous drip or intraperitoneal, subcutaneous, or intramuscular injection.

Formulations for topical administration may include but are not limited to lotions,
10 ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional
pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary
or desirable.

Compositions for oral administration include powders or granules, suspensions or
solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents,
15 flavorings, dispersing aids, emulsifiers or binders may be desirable.

Formulations for parenteral administration may include but are not limited to sterile
aqueous solutions which may also contain buffers, diluents and other suitable additives.

Dosing is dependent on the severity of the symptoms and on the responsiveness of the
subject to Halofuginone. Persons of ordinary skill in the art can easily determine optimum
20 dosages, dosing methodologies and repetition rates.

Example 8Methods of Treatment of Skin Disorders

As noted above, Halofuginone has been shown to be an effective inhibitor of the clinical
25 etiology of skin disorders, such as psoriasis and keloid and hypertrophic scar formation. The
following examples are illustrations only of methods of treating skin disorders characterized by
abnormal skin cell behavior with Halofuginone, and are not intended to be limiting.

The method includes the step of administering Halofuginone, in a pharmaceutically
acceptable carrier as described in Example 7 above, to a subject to be treated. Halofuginone is
30 administered according to an effective dosing methodology, preferably until a predefined
endpoint is reached, such as the absence of symptoms of a skin disorder in the subject. For
example, if a subject already had a keloid, the endpoint could be the reduction in size of the

keloid or its elimination.

Halofuginone can also be used as a pretreatment, administered to a subject before surgery to substantially prevent the formation of keloids or hypertrophic scars. Of course, such a pretreatment would be most effective for scheduled surgery, as that would allow Halofuginone to be administered for a sufficient period of time before surgery to be most effective.

Hereinafter, the term "treatment" includes both pretreatment, before a pathological condition has arisen, and treatment after the condition has arisen. For example, treatment of a keloid would include both administration of Halofuginone both before and after the genesis of the keloid. The term "treating" includes both treating the subject after the pathological condition has arisen, and preventing the development of the pathological condition.

Example 9

Method of Manufacture of a Medicament Containing Halofuginone

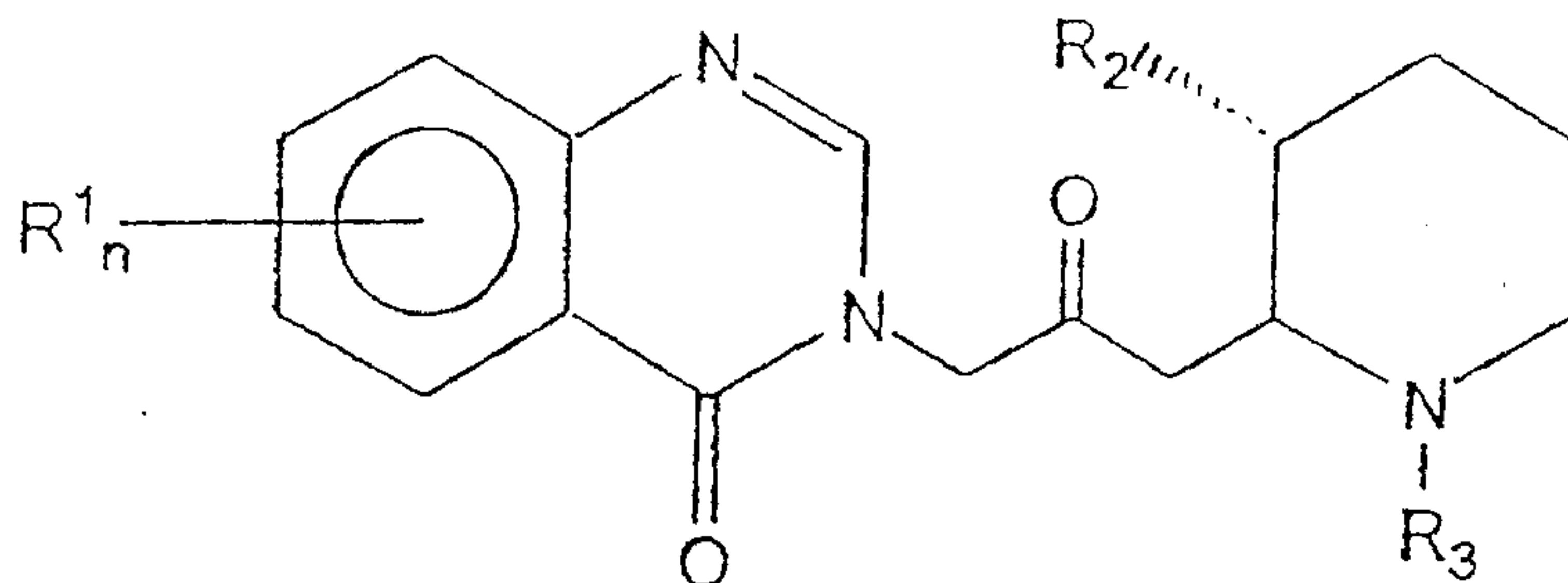
The following is an example of a method of manufacturing Halofuginone. First, Halofuginone is synthesized in accordance with good pharmaceutical manufacturing practice. Examples of methods of synthesizing Halofuginone, and related quinazolinone derivatives, are given in U.S. Patent No. 3,338,909. Next, Halofuginone is placed in a suitable pharmaceutical carrier, as described in Example 7 above, again in accordance with good pharmaceutical manufacturing practice.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and other applications of the invention may be made.

WHAT IS CLAIMED IS:

20

1. A composition for treating a skin disorder characterized by substantially abnormal cell behavior, comprising a pharmaceutically effective amount of a compound in combination with a pharmaceutically acceptable carrier, said compound being a member of a group having a formula:



wherein: $n=1$ or 2

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; and

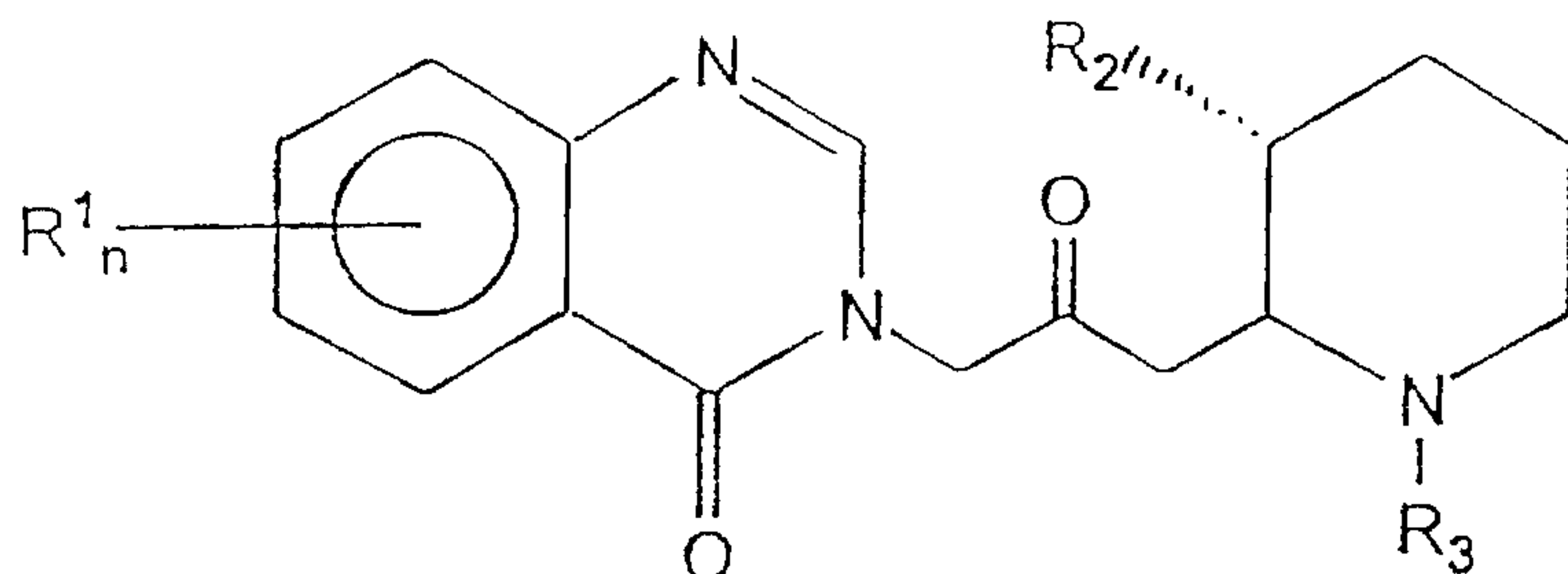
R_3 is a member of the group consisting of hydrogen and lower alkenoxy.

2. The composition according to claim 1, wherein said compound is Halofuginone.

3. The composition according to claim 1, wherein the skin disorder is selected from the group consisting of psoriasis, keloid, hypertrophic scar, acne, seborrhea and alopecia.

4. The composition according to claim 3, wherein the skin disorder is selected from the group consisting of psoriasis, keloid and hypertrophic scar.

5. A method for manufacturing a medicament for treating a skin disorder characterized by substantially abnormal cell behavior, comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, said compound being a member of a group having a formula:



wherein: $n=1$ or 2

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl.

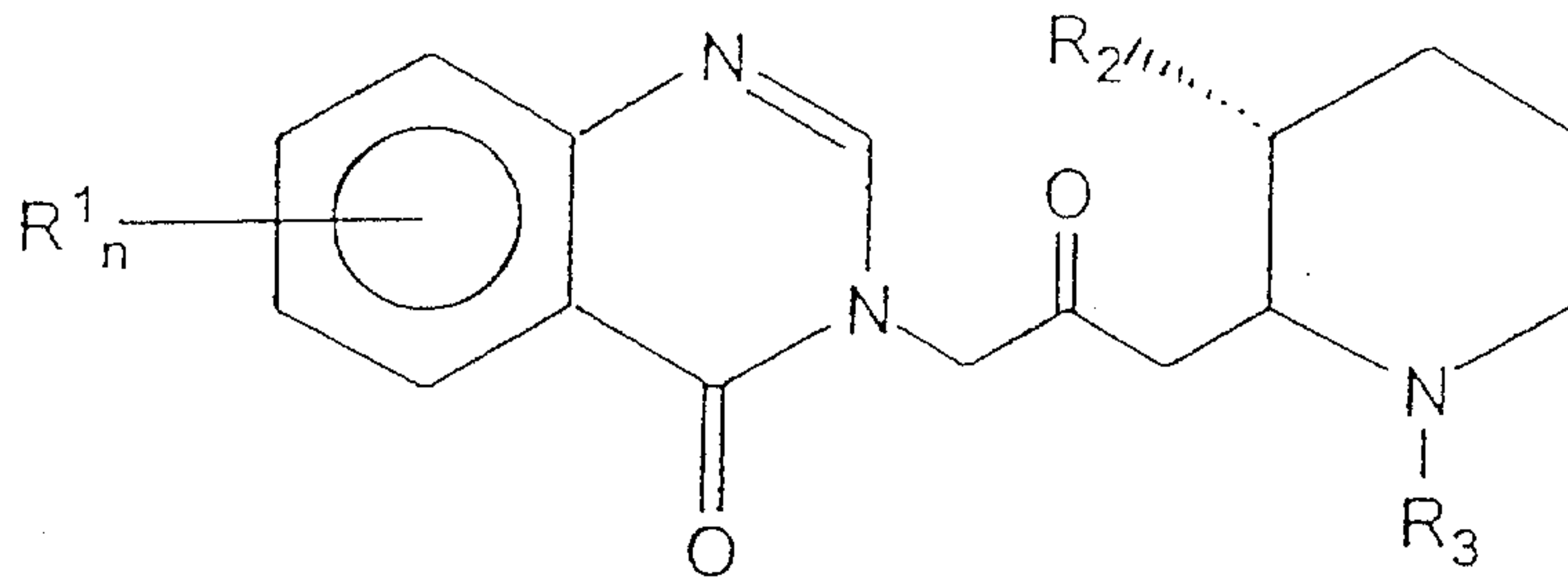
6. The method of claim 5, wherein said compound is Halofuginone.

7. The method of claim 5, wherein the skin disorder is selected from the group consisting of psoriasis, keloid, hypertrophic scar, acne, seborrhea and alopecia.

8. The method of claim 7, wherein the skin disorder is selected from the group consisting of psoriasis, keloid and hypertrophic scar.

9. A method of manufacturing a medicament for inhibiting formation of a keloid-like growth, the growth being caused by a performance of a surgical procedure,

the method comprising the step of placing a pharmaceutically effective amount of a compound in a pharmaceutically acceptable carrier, said compound being a member of a group having a formula:



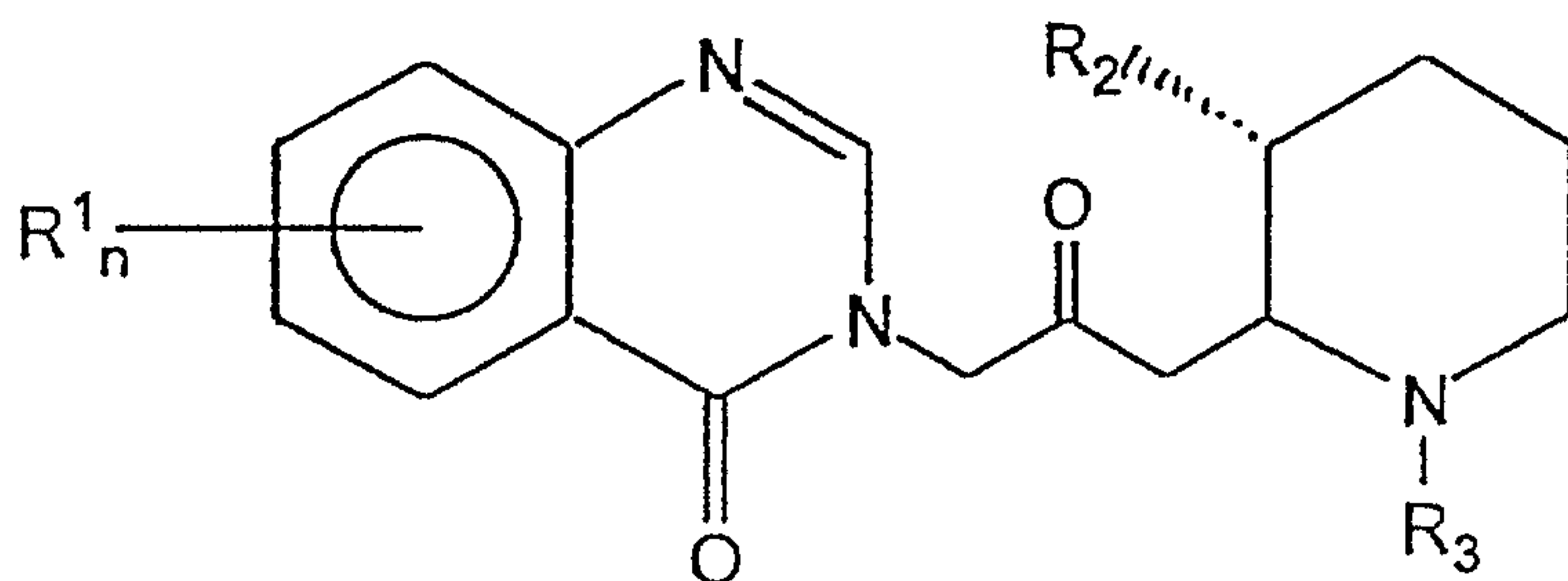
wherein: $n=1$ or 2

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy, and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl.

10. A composition for inhibiting formation of a keloid-like growth in a subject, the growth being caused by a performance of a surgical procedure on the subject, the composition comprising a pharmaceutically effective amount of a compound having a formula:



wherein: $n=1$ or 2

R_1 is a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl, and lower alkoxy;

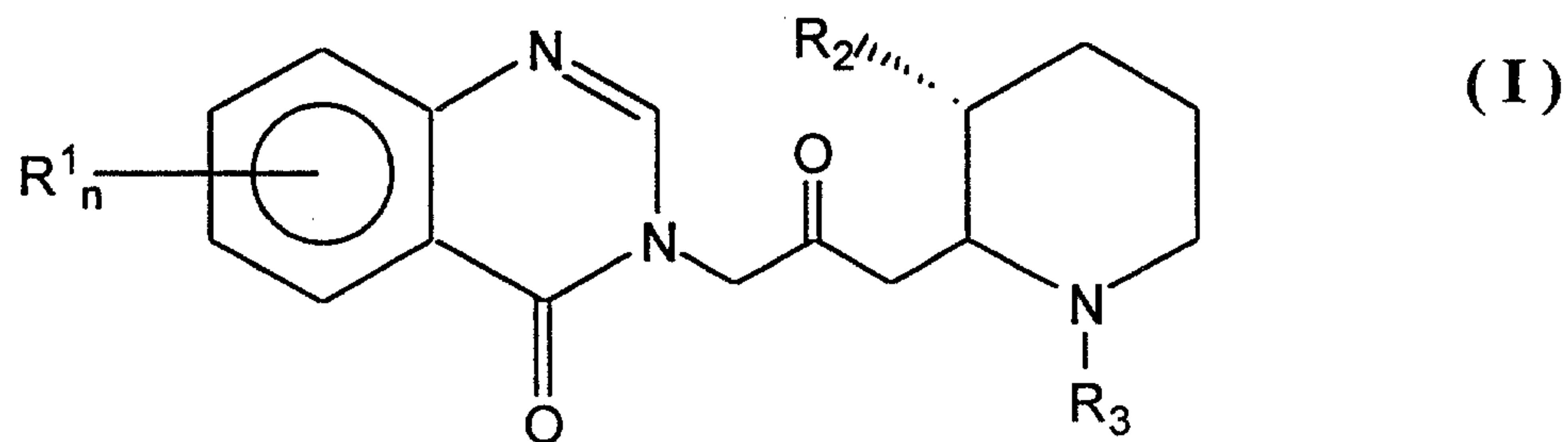
R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl.

11. The composition of claim 10, for administering to the subject substantially before the performance of the surgical procedure.

12. The composition of claim 10, for administering to the subject substantially after the performance of the surgical procedure.

13. A composition for prevention of formation of a scar, comprising a pharmaceutically effective amount of a compound in combination with a pharmaceutically acceptable carrier, said compound being a member of a group having a formula (I):



wherein: $n=1$ or 2

R_1 which at each occurrence is independently selected from the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy;

and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof.



FIG 1 B



FIG 1 A

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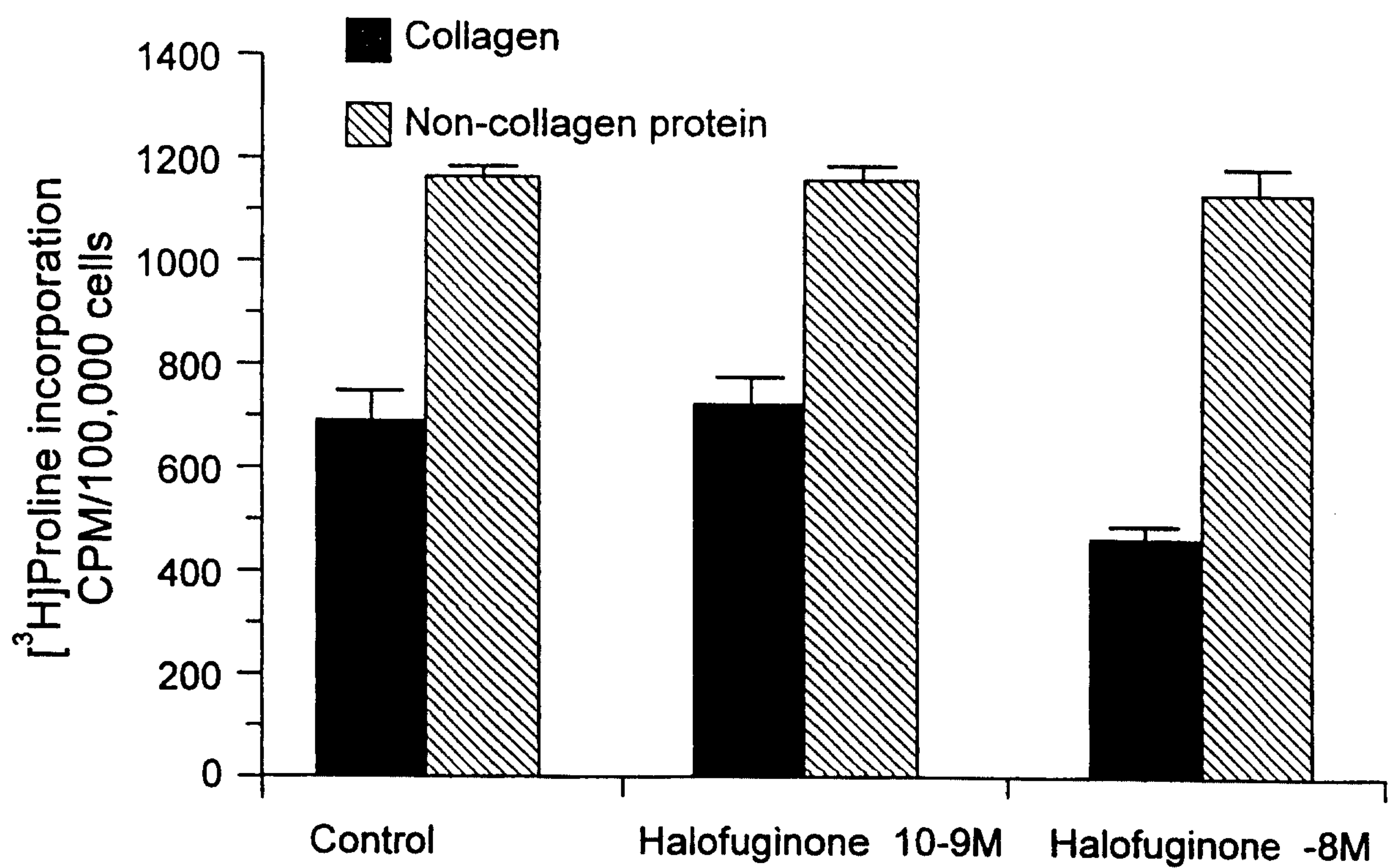


Fig. 2

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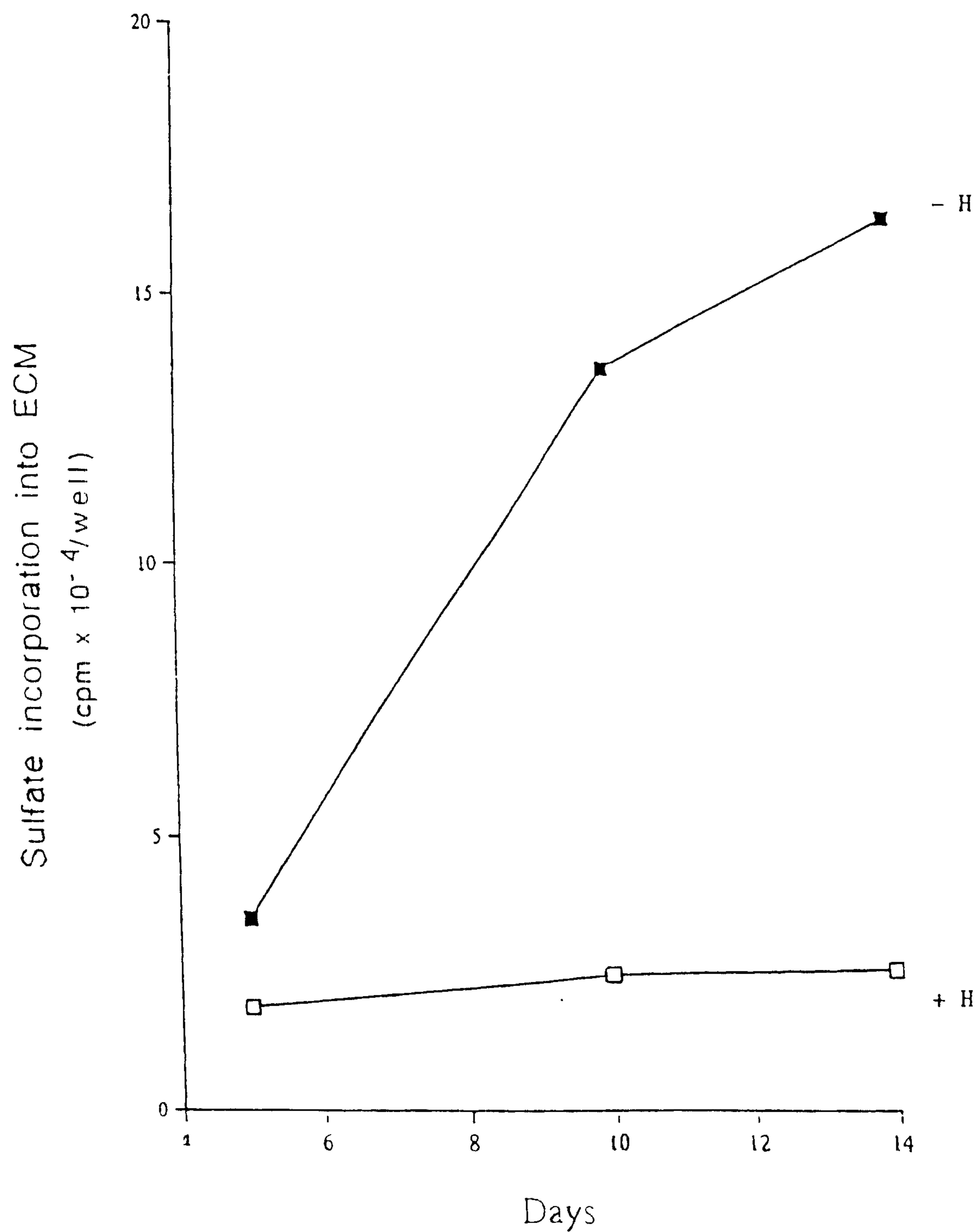
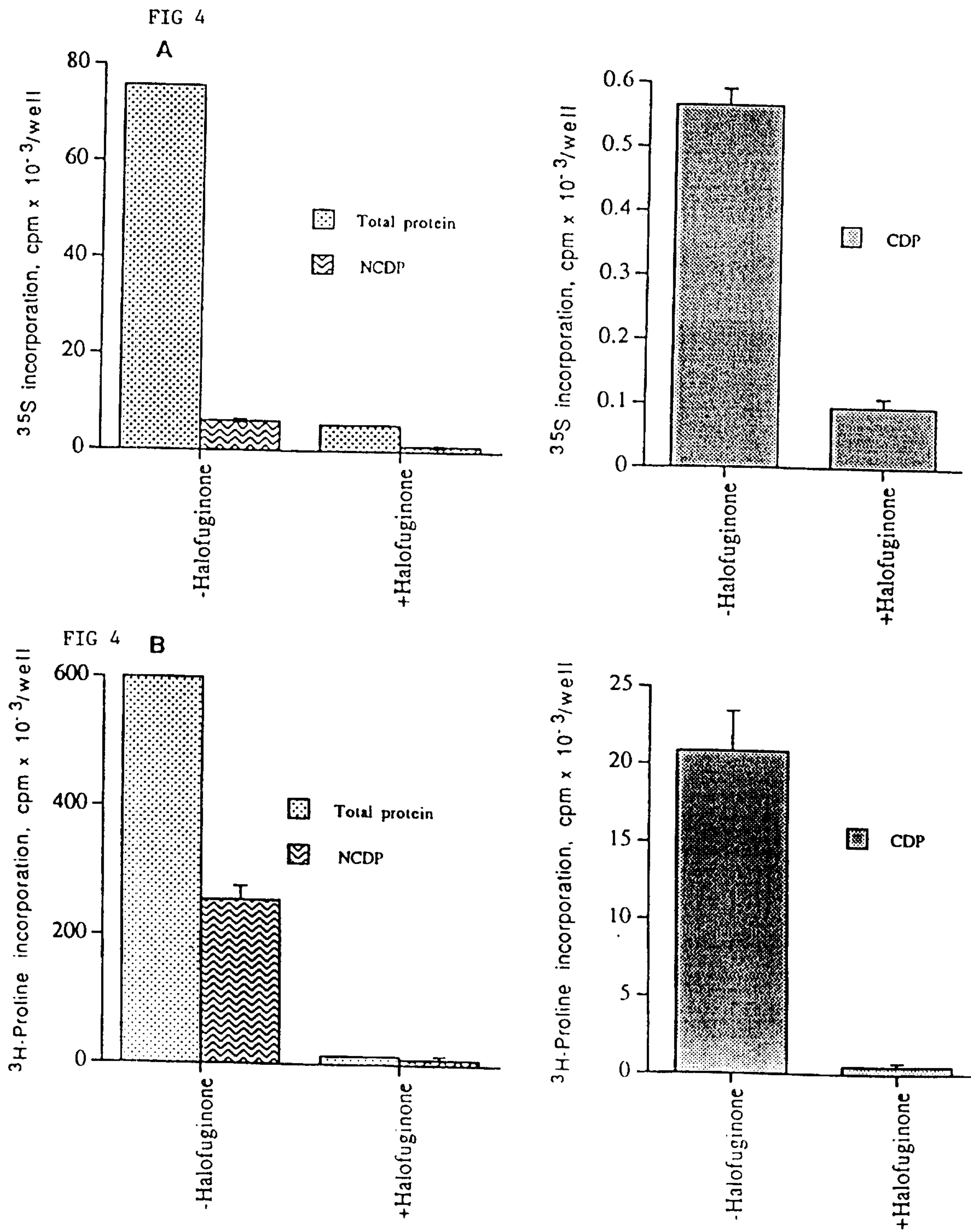
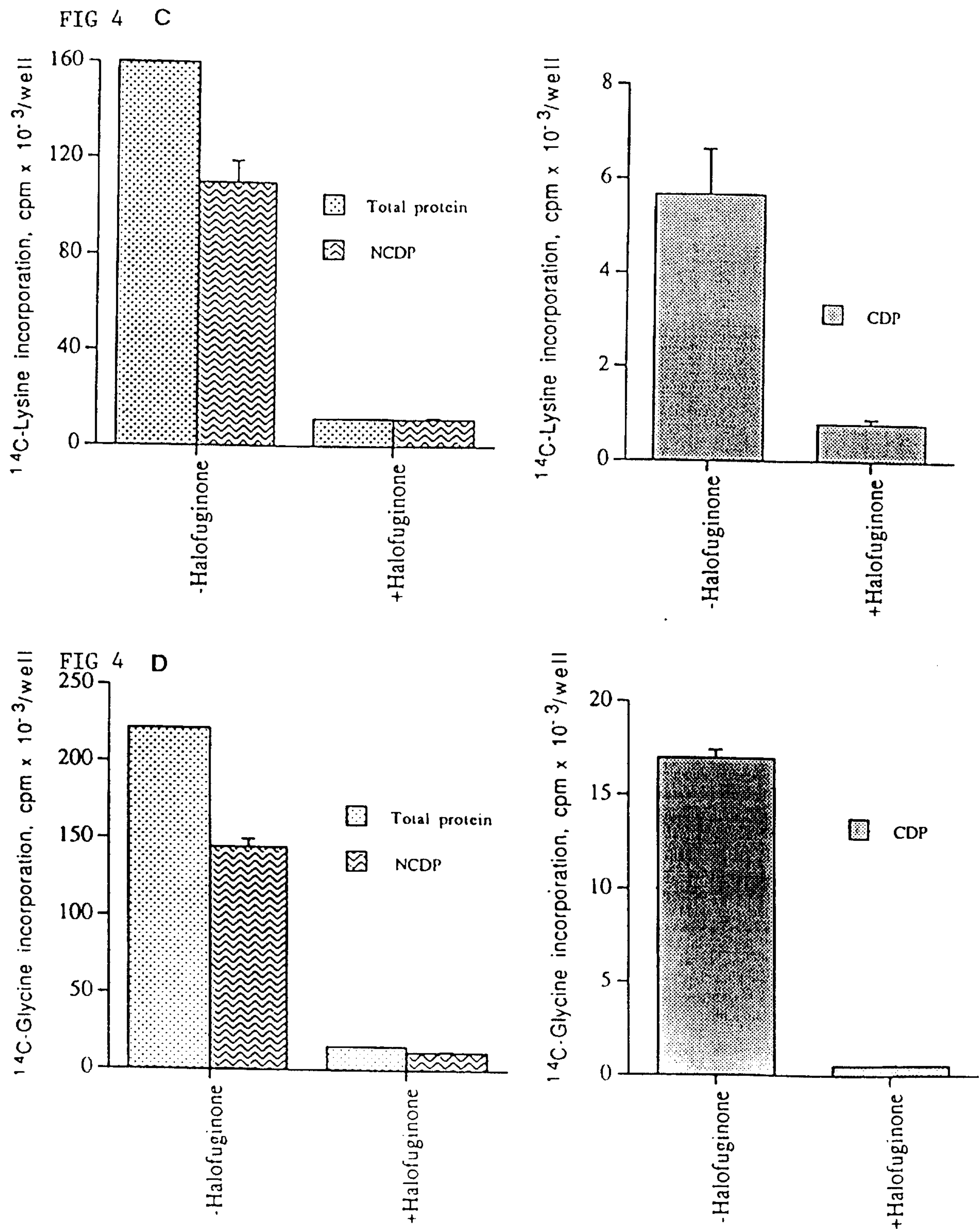


FIG 3

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Effect of halofuginone on ECM production
by corneal endothelial cells

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Effect of halofuginone on ECM production
by corneal endothelial cells

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Effect of halofuginone on ECM production by rat mesangial cells

