Wound Healing Agents: The Role of Natural and Non-Natural Products in Drug Development

Ângelo de Fátima[1,*], Luzia V. Modolo[2], Andréia C. Conegero Sanches[3] and Raphael R. Porto[1]

[1]*Grupo de Estudos em Química Orgânica e Biológica (GEQOB), Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, 31270-901, Brazil;* [2]*Plant Biology Division, The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK, 73401, USA.* [3]*Colegiado de Farmácia, Universidade Estadual do Oeste do Paraná, Rua Universitária, 2069, Bairro Faculdade , Cascavel, PR, 85819-110, Brazil*

Abstract: Impaired wound healing leads to infection and tissue necrosis. This has spurred the search for wound healing agents derived from natural and non-natural sources. Although natural products are widely used as lead compounds for the design of therapeutic drugs, few studies have looked for potential wound healing compounds in nature. In this review, we briefly discuss each phase of the wound healing process. Examples of natural and non-natural products with wound healing activities are listed, and the structure-activity relationship of fifty one compounds are described. An understanding of how these compounds exert their activities in biological systems is essential for their future development and application as wound healing agents.

Key Words: Wound healing agents, natural products, synthetic compounds.

INTRODUCTION

The isolation and structural characterization of natural products, along with studies on their potential as therapeutic agents, has brought new perspectives to the study of organic and medicinal chemistry. Natural products have been central to the development of front-line drugs [1], and are still the major source of innovative therapeutic agents for infectious diseases (both bacterial and fungal), cancer, lipid disorders and immunomodulation [2-5]. The structural complexity of these compounds can limit the scope of organic synthesis-driven chemical modifications necessary to optimize their therapeutic use. On the other hand, living organisms generally produce bioactive natural products in low amounts, thus limiting their use as a supply source for commercial drugs. The total synthesis of the potent anticancer natural product discodermolide, recently performed on a multigram scale [6], demonstrates that synthetic organic chemistry is a powerful tool for the development of both natural products of limited supply and those with very complex structures [7-8]. New developments in phytochemical analysis and organic synthesis are improving the discovery and access of new lead compounds with potential as drugs [9]. Organic synthesis can create more potent molecules (that may not be found in nature) from natural product prototypes.

THE WOUND HEALING PROCESS

The search for wound healing agents is one of the oldest challenges in medicine, and remains a challenge because the tissue repair mechanism is still not fully understood.

*Address correspondence to this author at the Grupo de Estudos em Química Orgânica e Biológica (GEQOB), Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG, 31270-901, Brazil; Tel: +55-31-3409-6373; Fax: +55-31-3409-5700;
E-mail: adefatima@qui.ufmg.br

Wound healing is a process in which the tissue affected by injury tries to repair the damage. The success of this effort depends on a complex physiological machinery that involves interactions between a variety of cells, biochemical mediators, and extra-cellular matrix molecules [10-11]. Loss of injured tissue is the first step in the re-establishment of cell hemostasis, where alterations occur in the physico-chemical composition at the injury site to establish blood coagulation (low O_2 tension, medium acidification, and increased reactive oxygen and nitrogen species). Chemical, physical, or biological stimuli activate neuronal, stromal, vascular, and circulatory system cells. This triggers a series of complex events that leads to inflammation. Secretion of signaling molecules by the immune system controls the wound healing process. Neutrophils play an important role in the tissue phagocytosis in the first 24 hours of injury [12]. In skin wounds, neighboring cells (epithelium, fibroblast, and keratinocyte) migrate in order to start fibroblast production followed by extracellular matrix deposition, angiogenesis, healing, and finally epithelium reconstitution [10]. Skin injury is classified according to its depth and width in the dermal tissue. Healing of partial-thickness wounds that occur superficially in the dermis normally does not lead to the formation of a visible scar. On the other hand, the healing process of deep-thickness injury (acute) triggers tissue remodeling and scar maturation [13].

A patient's age, nutritional condition, existence of systemic diseases (e.g. cardio circulatory, coagulation, and renal dysfunctions, diabetes, arteriosclerosis), lesion localization, and type of surgical procedure influence the wound healing process [14,15].

PHASES OF WOUND HEALING

Wound healing is didactically classified in five phases to facilitate the understanding of such a complex process (Fig.

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1). These phases include blood coagulation, inflammation, cell proliferation, lesion contraction, and remodeling that occur simultaneously to promote an efficient healing [14,15].

Coagulation Phase

Alteration in the tissue's integrity caused by the injury triggers immediate vessel constriction resulting in blood clot and platelet aggregation that limit further blood loss [13]. Secretion of growth factors (insulin-like growth factor 1, epidermal growth factor, transforming growth factor-β, and platelet factor-IV) is driven by platelet degranulation, where these proteins induce the activation of fibroblasts, endothelial cells, and macrophages. This cascade is activated to rescue the normal hemostasis strictly in cases where the lesion undergoes hemorrhage.

Inflammation Phase

This phase is responsible for combating the invasion of microorganisms through the coordinated action of neutrophils, macrophages, and lymphocytes. A cascade of events is activated concomitantly with coagulation and starts with polymorphonuclear leukocytes infiltrating the wound. These neutrophil granulocytes are attracted by fragments of extracellular matrix protein, transforming growth factor-β, complement components (C3a, C5a), and formyl-methionyl peptide products from bacterial infection [13]. Besides the induction of phagocytosis, macrophage migration generates reactive oxygen (e.g. superoxide anion, O_2^-) and nitrogen (e.g. nitric oxide, NO) species that play important roles in the wound healing [12]. Macrophages are also the link in both proliferative response and production of proteolytic enzymes (e.g. collagenase) that help to debride the wound. Epithelial cell proliferation continues to form a thickened epidermal covering layer.

Proliferative Phase

This phase normally starts three-day post-injury and may last for two-four weeks. The proliferative phase is characterized by fibroblast migration followed by angiogenesis and re-epithelization [13,16]. Fibroblasts migrate to the site of injury and induce the production of collagen to substitute for the damaged tissue, a process called fibroplasia. Fibroplasia combines the recruiting of macrophages, fibroblasts, and neovascular matrix components (e.g. fibronectin, glycosaminoglycan and collagen) leading to the formation of the matrix. Proteases are also released during the matrix formation to remove foreign bodies and dead tissue [17]. Growth factors stimulate angiogenesis that is responsible for increasing the oxygen content and nutrients in the affected tissue to promote its regeneration [14,15,18]. In wounded skin, migration and proliferation of keratinocytes promotes epithelium differentiation and reorganization of the membrane that connects epidermis and dermis.

Wound Contraction and Remodeling

The contraction phase occurs to reduce the area of damage since the affected tissue is about to be completely regenerated. Wound size can be decreased by 20% and up to 62% in injuries of total and partial thickness, respectively [14,15]. Modeling is the last phase of wound healing and comprises the scar formation (also called cicatrizing process). This phase may last for several days or even months and provokes an increase of the tension forces and reduction of erythema and scar size. The extracellular matrix starts changing its composition, and reduction in macrophage and fibroblast density and growth factor migration take place. The metabolism of neovascular tissues is also decreased to make the scar avascular with the formation of a flat surface [16].

WOUND HEALING AGENTS

Natural Products and Derivatives as Wound Healing Agents

Terpenoids (also called isoprenoids or isopentenoids) are secondary metabolites assembled from the basic five-carbon-unit isoprene with extraordinary diversity in the plant kingdom. Members of this class include monoterpenoids (two

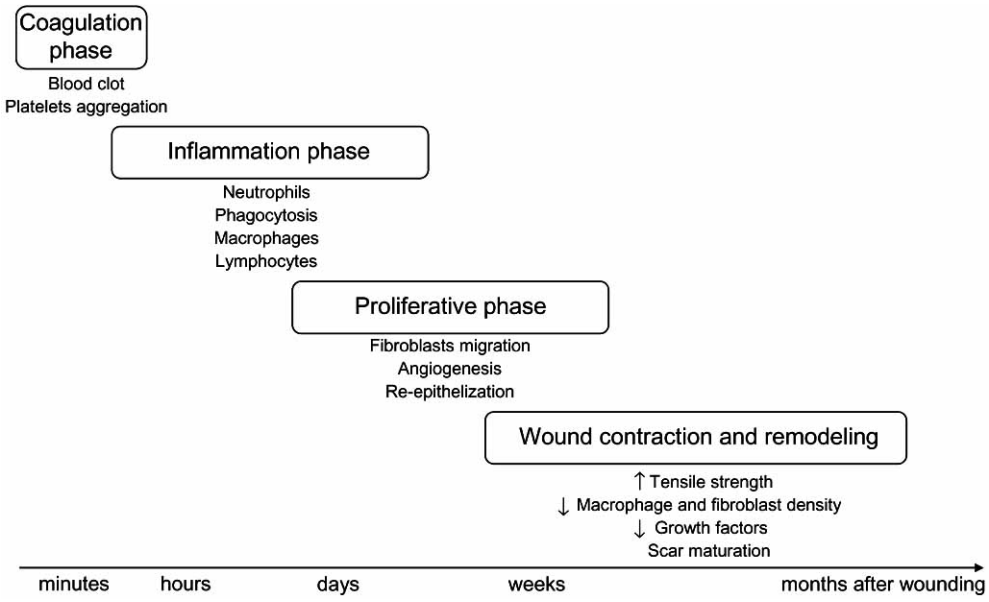


Fig. (1). Schematic representation of the events that take place during the wound healing process.

isoprene units), sesquiterpenoids (three isoprene units), diterpenoids (four isoprene units), sesterterpenoids (five isoprene units), triterpenoids (six isoprene units), carotenoids (eight isoprene units), polyprenoids (ubiquinone side chain and rubber). Terpenoids are widespread small molecular weight compounds of which over 20,000 have been identified [19].

Asiatic acid (**1**) (Fig. **2**) and its derivatives are some of the most promising terpenoids for the development of wound

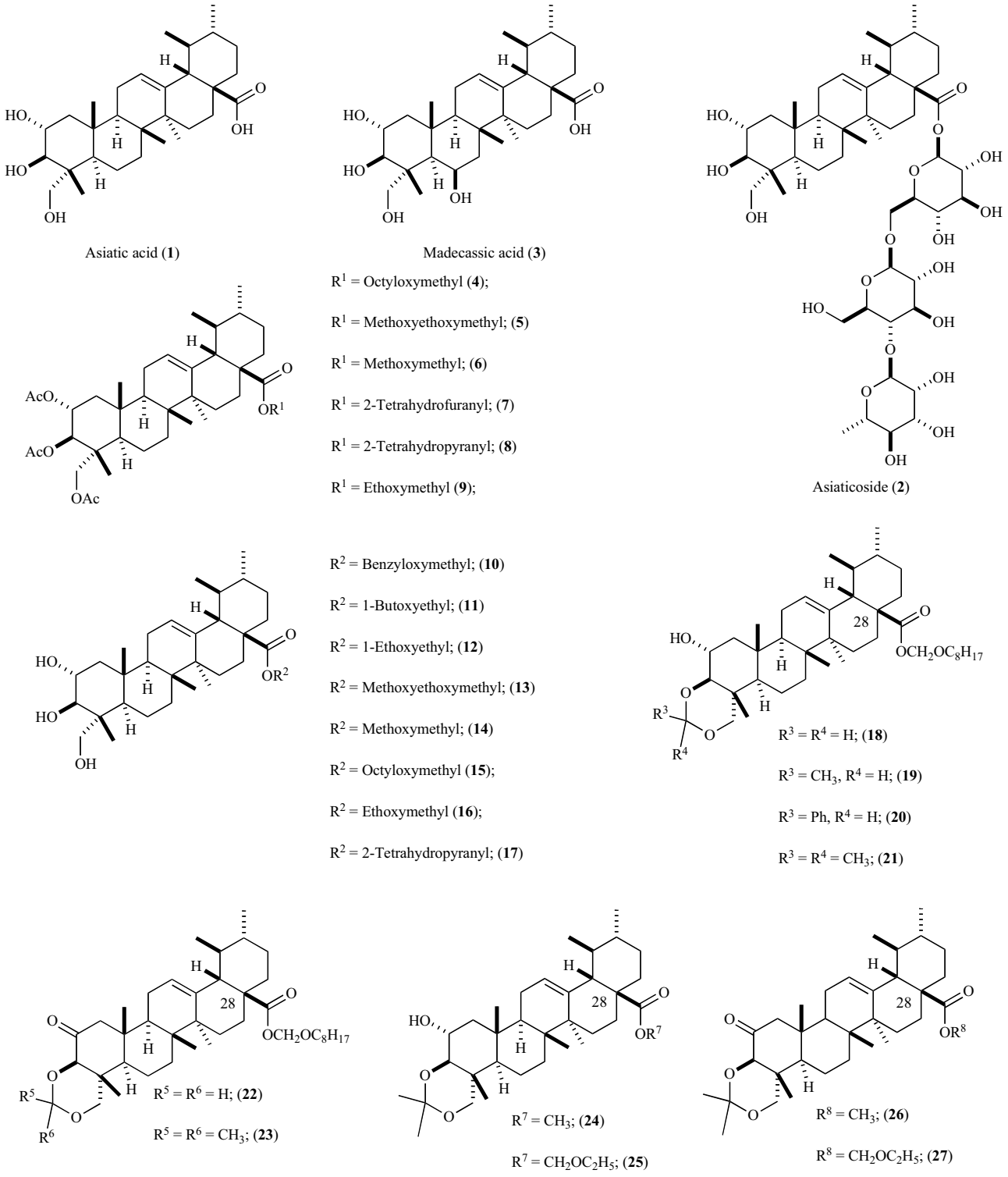


Fig. (2). Structure of terpenoid compounds and derivatives tested for wound healing activity.

healing agents. Asiatic acid (**1**), madecassic acid (**2**), and asiaticoside (**3**) (Fig. **2**) are the major terpenes synthesized by *Centella asiatica* (L.) Urban [20]. *C. asiatica* extract has been used by diverse ancient cultures and tribal groups as a wound healing agent [21,22]. The wound healing property led to the commercialization of this extract under the trade name Madecassol® [23,24]. Topical applications of Madecassol® on the injured area of skin results in a decreased granulation and scar formation and an increased skin tension strength, features that prevents sequelae from inflammatory responses [25,26]. Asiatic acid (**1**) is the most active healing agent present in *C. asiatica* extracts being responsible for the stimulation of collagen synthesis [27,28]. Studies suggest that the therapeutic effect of asiaticoside (**3**) is associated with its *in vivo* deglycosylation yielding asiatic acid (**1**) [26,29].

The first structure-activity relationship studies performed with asiatic acid (**1**) and its derivatives in the wound healing process was reported in the last decade using rats at the seventh postoperative day [30]. The carboxylic and hydroxyl groups in the asiatic acid (**1**) delay its permeation into the dermis. Derivatives **4**-**17** (Fig. **1**) were synthesized from asiatic acid (**1**) and asiaticoside (**3**) in an effort to improve the asiatic acid (**1**) dermal permeability. Tensile strength was the parameter used for determining the wound healing activity of these compounds. Tensile strength corresponds to the longitudinal stress the tissue can bear without tearing apart. Asiatic acid (**1**) treatment promoted a tensile strength of 336 ± 19 g/cm^2, and modifications in the structure of this terpenoid did not affect its wound healing properties, except for the ethoxymethyl derivative (**9**) whose activity was enhanced (tensile strength = 487 ± 32 g/cm^2). Acetylation of hydroxyl groups in asiatic acid (**1**) should improve its bioavailability as the hydroxyl groups of compounds **1**, **2**, and **3** are known to be metabolized through oxidation or conjugation reaction [31]. Replacement of the glycosyl moiety by less bulky or less polar groups negatively affected the activity of asiaticoside (**3**). The potency of derivatives **10**-**14** was 13-30% lower than that for asiaticoside (**3**) [30]. However, the incorporation of much more bulky or polar groups (derivatives **15**-**17**) conferred potency comparable to that for asiaticoside (**3**). Ketal asiatic acid derivatives were reported as anti-inflammatory and wound healing agents [32,33]. The synthetic ketal asiatic derivatives **18**-**27** (Fig. **2**) were evaluated for wound healing activity by measuring the tensile strength in skin strips from injured segments. Injured skins treated with ketal derivatives **18**-**21** presented slightly higher tensile strength (311-337 g/cm^2) when compared to untreated injured skins (305 g/cm^2). The dimethylketal derivative **21** improved the tensile strength of injured tissues by 10%. The wound healing capacity was clearly increased by the presence of bulky esters at C28: methoxyoctyl ester (tensile strength = 337 g/cm^2); methoxyethyl ester and ethyl ester (365 g/cm^2). The conversion of hydroxyl group at C22 to a ketone moiety did not affect significantly the wound healing activity. The time necessary to heal a wound by 50% (HT_{50}) was not significantly different in injured tissues treated with the ketal derivatives **18**-**20** when compared to untreated ones. In contrast, wounded tissues treated with the derivative **21** healed faster (HT_{50} = 5.7 days) than the untreated injured tissues (HT_{50} = 6.2 days) [33]. These results indicate that **21** is an interesting lead compound for wound healing drug design.

Oleanolic acid (**28**; Fig. **3**), obtained from the acid hydrolysis of *Anredera diffusa* (Basellaceae) extract was found to be a potential wound healing agent [34]. It was highly efficient when topically administrated in injured mice at 40 μg/g body weight. Wounds treated with oleanolic acid (**28**) at this dose healed 39% faster than untreated wounds [34].

(+)-*Epi*-α-bisabolol (**29**; Fig. **3**) is the terpene responsible for the *in vivo* wound healing activity of *Peperomia galioides* (Piperaceae) extracts [35]. Vilegas and coworkers (2001) tested other terpenols that are commercially available [α-bisabolol (**30**), α-terpineol (**31**), *trans*-nerolidol (**32**), and *trans*,*trans*-farnesol (**33**)] for wound healing activity (Fig. **3**). The main constituent of *Matricaria recutita* (Asteraceae) is α-bisabolol (**30**). Guinea pigs wounded by exposure to ultraviolet light were treated with compounds **29**-**33** and the tensile strength of injured tissues was evaluated. (+)-*Epi*-bisabolol (**29**) presented an effective dose for increasing the tensile strength by 50% (ED_{50}) of 155 μg/g body weight. Both α-bisabolol (**30**) and α-terpineol (**31**) also promoted tensile strength of injured tissue at ED_{50} values of 228 and 240 μg/g body weight, respectively. The acyclic-related terpenols *trans*-nerolidol (**32**) and *trans*,*trans*-farnesol (**33**) had no effect on tensile strength. Tensile strength of injured tissue *per se* can be a misleading parameter for the determination of wound healing because tensile strength can also be increased from the death of cells. The toxic effects of compounds **29**-**31** were then evaluated on mouse embryonic fibroblast (3T3) cells [35]. The concentration of compounds **29**-**31** necessary to inhibit 3T3 cells growth by 50% (GI_{50}) was much lower (8.0, 84, and 17 μg/mL, respectively) than the effective dose of these compounds to induce tensile strength. These results indicate that the terpenols **29**-**31** increase the tensile strength of injured tissue by killing the cells instead of promoting wound healing.

Saponins are glycosylated triterpenoid, steroid or steroidal glycoalkaloids plant metabolites [36]. This class of plant secondary metabolites is promising for drug design in many therapies. The oral administration of red ginseng root *Panax ginseng* (Araliaceae) in patients in clinical trials for diabetes mellitus and Werner's syndrome treatments stimulated the repairing of their intractable skin ulcers. Also, the local administration of saponins considerably improved the cicatrizing process in diabetic or aging rats [37,38]. The term "*Panax*" derives from Greek "*pan*" and "*akos*", which means "all healing". Six ginsenosides (compounds **34**-**39**; Fig. **3**) with high wound healing activity were isolated from the red ginseng root [39]. Rb_1 (**34**) exhibited the strongest burn wound healing action being potent at a concentration as low as 10 fg/g ointment [39]. Rb_1 (**34**), when applied at 100 fg to 1 ng per wound, increased both revascularization in the surrounding injured tissue and production of vascular endothelial growth factor (VEGF) and interleukin (IL)-1-β from the burn wound [39]. No significant difference was observed between mice treated with the ginsenosides **35** or **37**-**39** and untreated mice. The ginsenoside Rc (**36**), however, presented wound healing activity at 1 ng/g ointment [39].

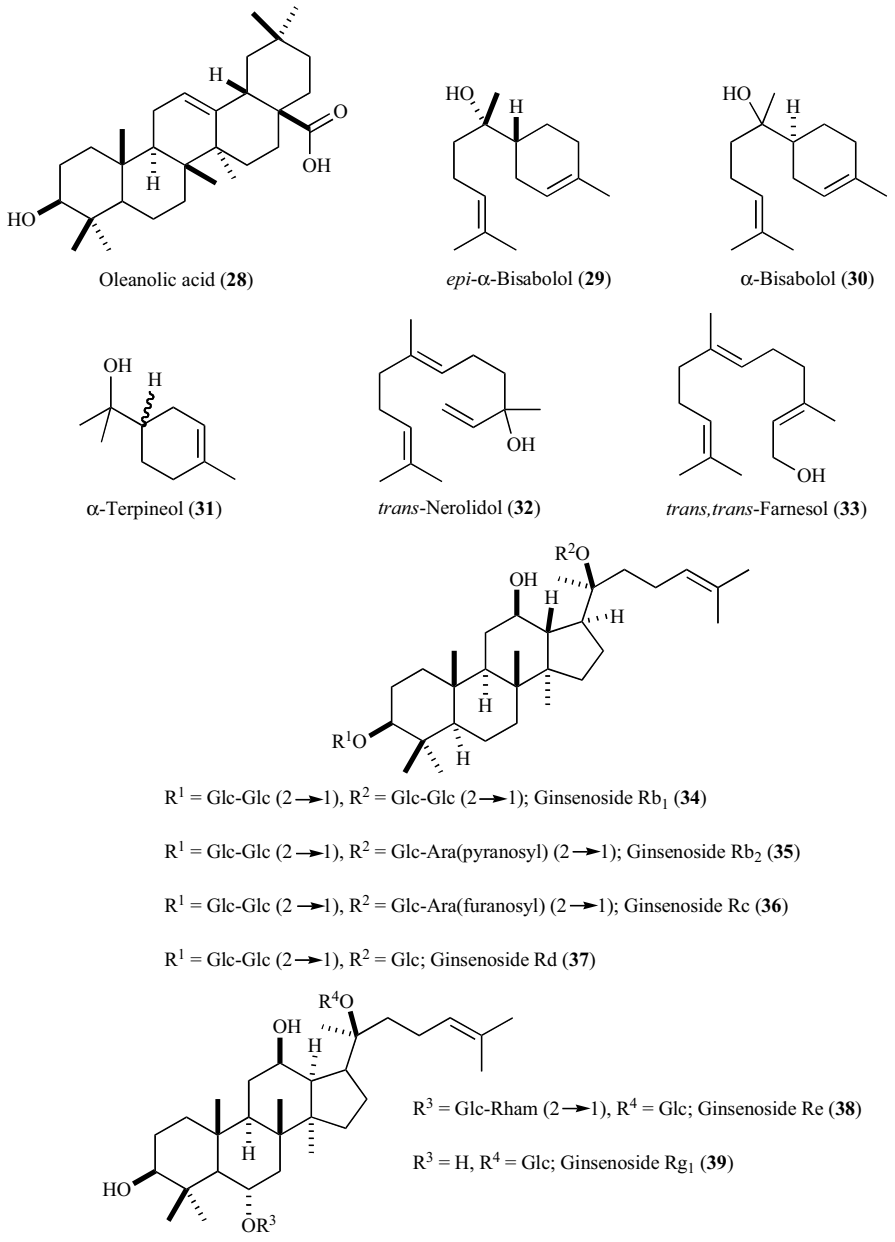


Fig. (3). Structure of oleanolic acid, terpenols, and ginsenosides saponins tested for wound healing activity. Glc, glucose residue; Rham, rhamnose residue.

Quinones are compounds ubiquitous in all living organisms. They are widely used as anticancer, antibacterial, antifertility, antiprotozoa and as antifungal agents [40-42]. Embelin (**40**; Fig. **4A**) is a quinone obtained from the ethanolic extract of fruits and leaves of *Embelia ribes* (Myrsinaceae) [43-45]. Embelin (**40**) exhibited strong wound healing activity when tested on an excision wound model. Embelin (**40**) caused wound closure by 89.3% and 98.5% in injured animals after 12 and 16 days of treatment. The crude ethanolic extract of *Embelia ribes* also provided similar results at the same period of treatment (85.2% and 97.0%, respectively). Non-treated animals exhibited wound closure by 68.2% to 85.3%, between the 12th and 16th day of experiment [45]. The complete re-epithelization of the excision wound occurred within 18.2 days for embelin (**40**)-treated animals, 18.7 days for crude ethanolic extract-treated animals, and 20.3 days for untreated animals. The effects of embelin (**40**) on the complete re-epithelization and tensile strength of the incision wound were comparable to those for the positive drug control framycetin [45]. Histological studies also revealed the beneficial effects of embelin (**40**) when orally administrated to the animals. Acute inflammation, fibroblastic connective tissues, and a few blood vessels were observed in cells from untreated animals while embelin (**40**)-treated animals showed complete healing, with increased collagen formation and a number of new blood vessels [45].

Roots of the Chinese herb *Rheum officinale* (Polygonaceae) are traditionally used in wound healing. Anthraquinone emodin (**41**; Fig. **4A**) was recently isolated from *Rheum officinale* roots and exhibited cicatrizing activity [46]. The rate of wound contraction was 41.6% five days after the treatment of injured rats with 400 μg/mL emodin (**41**) whereas the rate of wound contraction for untreated rats was only 21.6% [46]. This difference was even higher 7 days post-treatment where wounds were contracted by 56.1, 63.1, and 80.5% in animals treated with emodin (**41**) at 100, 200 and 400 μg/mL, respectively. Under these conditions, non-treated wounds were contracted by 47.3% only. Wound contraction is a reliable parameter to evaluate cicatrizing activity, and corresponds to the centripetal movement of the edges of a full-thickness wound to promote the closure of the defect [47].

Alkaloids are a diverse group of over 12,000 nitrogen-containing secondary metabolites produced by approximately 20% of existing plant species. Taspine (**42**; Fig. **4A**) is the main alkaloid component of dragon's blood, a red viscous latex extracted from the cortex of several plant species belonging to the family Euphorbiaceae. This latex is used in folk medicine in South America as a cicatrizing agent and for several other purposes [48,49]. Taspine (**42**) accounts for the cicatrizing activity of extracts of *Croton lechleri* [50,51]. Commercial taspine hydrochloride at 0.1 mg/mL had significant cicatrizing activity provoking a wound-breaking strength of 59.2 ± 11.3 g in mice. The commercial compound was more effective than dragon's blood when applied at concentration of 10% v/v (wound-breaking strength = 71.4 ± 8.4 g). At this concentration, dragon's blood presents the equivalent to 0.1 to 0.2 mg/mL of taspine (**42**) [50]. The wound-breaking strength value for the untreated animals was 45.2 ± 8.3 g. The ED_{50} value for taspine hydrochloride was determined as 375 μg/Kg mice body weight [50].

Histological studies report other natural products and/or derivatives with wound healing properties [52-59]. Living organisms are a storehouse for an array of lead compounds for drug design, in particular, wound healing agents.

Non-Natural Compounds as Wound Healing Agents

Organic synthesis is the cornerstone upon which pharmaceutical and medicinal chemistry is built [60]. The development of new synthetic methods is helping the discovery and access to new lead compounds; making organic chemistry one of the main drivers in drug design [9].

The wound healing effects of 2-benzazepine derivatives (**43**) and (**44**) (Fig. **4B**) were recently reported [61]. Benzazepine consists of a benzene ring fused to an azepine ring. Many compounds that contain this structure moiety interact with biogenic amine receptors, conferring to them a variety of pharmacological effects including antipsychotic and anticancer activities [62,63]. The compound (**43**) decreased the wound area in mice by 76, 54, 32, and 21% after two, four, six, and eight days of treatment, respectively. Differences

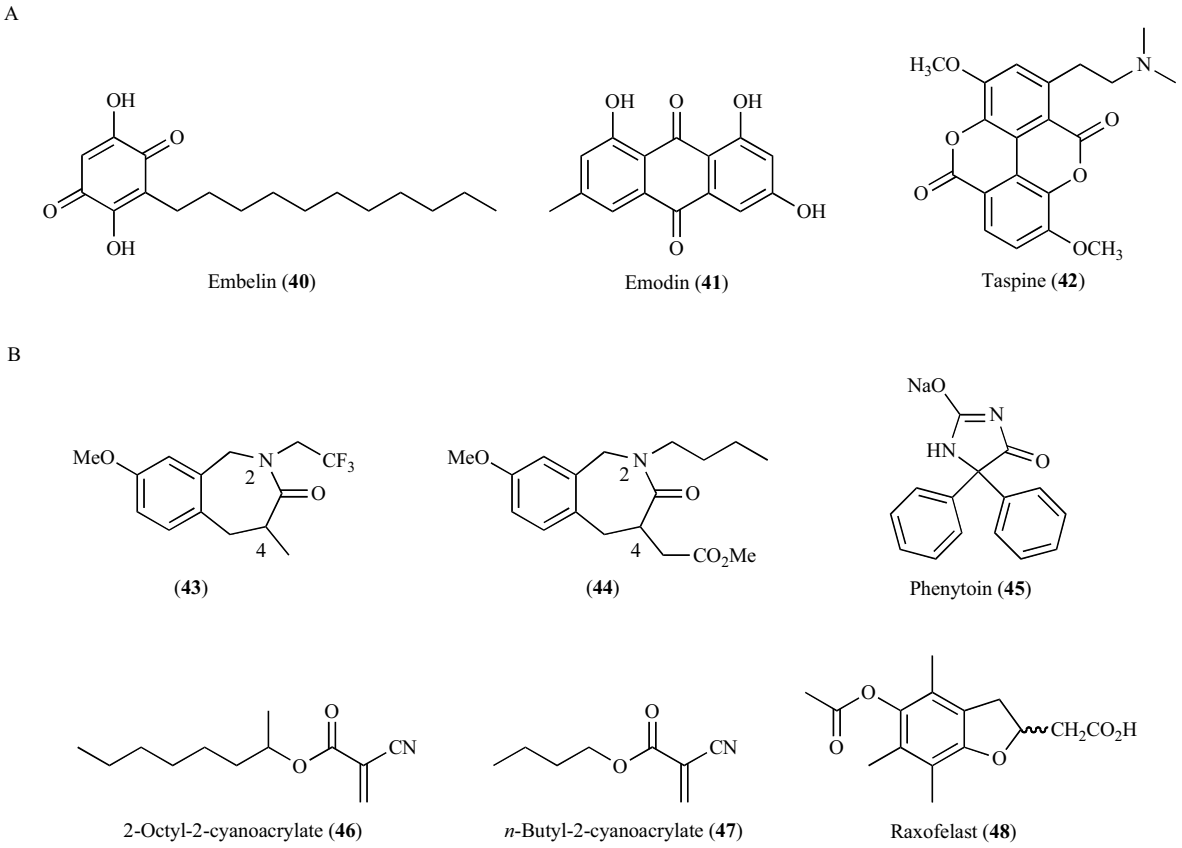


Fig. (4). Structure of natural (A) and non-natural compounds (B) with wound healing activity.

between treated mice and non-treated ones were statistically significant six and eight days post-injury. Topical application of benzazepine derivative **44** did not affect the wound closure [61]. These results suggest that the functional groups at N2 and C4 play important roles in the wound healing activity of compound **43**. The synthesis of more derivatives would be valuable to determine the structural requirements for the wound healing activity of benzazepines.

Phenytoin (**45**; Fig. **4B**), also known as sodium diphenylhydantoin, is an antiepileptic drug used clinically since 1938 [64]. Many beneficial effects of phenytoin (**45**) on various types of lesions have been reported, including skin ulcers, burns, and periodontal diseases [65-67]. Despite its activity toward many types of lesions, the role of phenytoin (**45**) in wound healing is not fully understood. Its potential as a wound healing agent was first assessed by Kimball and Horan [68] from the induction of gingival hyperplasia in patients treated with phenytoin (**45**). Oral use of phenytoin (**45**) was also reported in clinical studies for cutaneous wound healing in 28 patients with venous stasis ulcers [69]. The mean-wound area in phenytoin (**45**)-treated patients decreased by 0.65 cm^2 after 13 weeks of treatment while untreated patients had the lesion increased by 7.7 cm^2 over the same period of time.

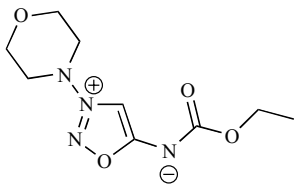
Phenytoin (**45**) has also been examined in the treatment of foot ulcers in diabetic patients [70]. Mean healing time was 21 days in the phenytoin (**45**)-treated patients, while in the untreated ones the mean healing time was 45 days. The effect of phenytoin (**45**) on skin wound healing using a rat model was also investigated [71]. The average tensile strength (measured as the tissue break force) of the phenytoin (**45**)-treated wound was 0.5 ± 0.1 MPa and the untreated ones presented an average tensile strength of 0.02 ± 0.01 MPa. Moreover, histological analysis of the phenytoin (**45**)-treated group showed a large amount of fibroblast proliferation, collagen synthesis and neovascularization. All wounds that received phenytoin (**45**) applications were completely healed 6-week post-operation, while 1% of the wound still remained open in untreated animals [71]. Overall, the wound healing properties of phenytoin (**45**) toward many types of lesions points to it as a lead compound for the design of new wound healing agents. Studies evaluating the phenytoin (**45**) molecular targets will be very important for understanding its mechanism of action.

Cyanoacrylate is the generic name for the compounds 2-octyl-cyanoacrylate (**46**) or *n*-butyl-cyanoacrylate (**47**) (Fig. **4B**). Both compounds are used in medical glues under the trade names Dermabond® and Traumaseal®. Compounds **46** and **47** are also employed as liquid bandage and wound adhesives. They have been shown to enhance the cicatrizing process by reducing the edema, the amount of granulation tissue, and the scab formation [72,73].

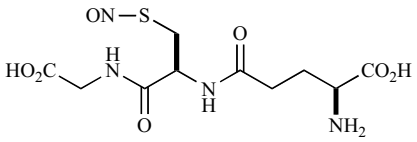
Raxofelast (**48**; Fig. **4B**), a hydrophilic vitamin E-like antioxidant, stimulated wound healing in mice and inhibited lipid peroxidation with improvement of wound healing in experimental burn wounds [74,75]. Its inhibitory effect on lipid peroxidation comes from its *in vivo* deacetylation generating an effective scavenger of reactive oxygen species [76,77]. The effects of raxofelast (**48**) in impaired wound healing were reported from studies with an incisional skin-wound model produced on the back of female diabetic C57BL/KsJ db+/db+ mice [75]. The administration of raxofelast (**48**) did not alter the process of wound repair in normal mice, but significantly improved the wound healing in diabetic mice whose cicatrizing ability is impaired. Administration of raxofelast (**48**) triggered the stimulation of angiogenesis, re-epithelization, synthesis and maturation of extracellular matrix [75].

Nitric oxide (NO) is a gaseous free radical that plays important roles in living organisms and is claimed as a potent wound healing agent. In animals, NO originates from the oxidation of L-arginine to L-citruline in a complex process catalyzed by nitric oxide synthase (NOS) enzymes. Nicotinamide adenine dinucleotide (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin (H_4B), and calmodulin can all function as cofactors for NOS activity [78]. The enzymes nNOS (or type I NOS), iNOS (or type II NOS), and eNOS (or type III NOS) are the three main isoforms that were first identified in brain tissues, activated macrophages, and vascular endothelium, respectively [79-85]. The beneficial effects of NO for the wound repair may be attributed to its functional influences on angiogenesis, inflammation, cell proliferation, matrix deposition, and remodeling [86]. L-arginine, the substrate for nitric oxide synthase (NOS), was identified in 1978 as a wound healing enhancer [10]. Accumulating evidence indicates that NO plays a key role in the wound repair of normal animals [87-89]. Production of nitrite (NO_2^-) and nitrate (NO_3^-), the stable NO metabolites, is elevated early in the fluid of subcutaneous wounds [88]. All isoforms of nitric oxide synthase (NOS) were shown to be involved in the wound healing process where mRNA and/or protein expression were increased in cutaneous wounds [89-91]. Moreover, application of NOS competitive inhibitors to the wound surface decreased collagen deposition and broke the strength of incisional wounds, which led to an impaired healing [87,92]. NO gas as well as NO donors have been shown to increase the wound healing process [93-97].

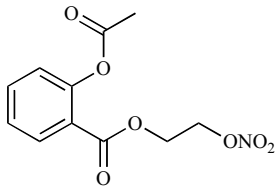
Molsidomine (**49**; Fig. **5**) is an NO donor that significantly increased the fresh wound breaking strength in diabetic wounded rats from 184 ± 12 g (non-treated diabetic rats) to 258 ± 33 g (treated diabetic rats). Molsidomine (**49**) did not affect the wound breaking strength in normal wounded rats (417 ± 25 g in molsidomine (**49**)-treated animals *versus* 409± 33 g in non-treated animals) [93]. Hydroxyproline (OHP) content significantly increased upon subcutaneous implant of polyvinyl alcohol (PVA) sponges containing molsidomine (**49**) in both normal and diabetic wounded animals. Molsidomine (**49**)-treated animals contained 1645 ± 82 μg OHP/100 mg sponge while non-treated animals presented 1311 ± 105 μg OHP /100 mg sponge. Diabetic animals treated with molsidomine (**49**) presented OHP content of 902 ± 131 μg/100 mg sponge and non-treated diabetic animals exhibited 555 ± 96 μg OHP/100 mg sponge [93]. OHP formation is directly correlated with increasing of the collagen levels and the healing process depends on the regulated biosynthesis and deposition of new



Molsidomine (**49**)



S-Nitrosoglutathione (**50**)



NOE-Aspirin (**51**)

Fig. (5). Structure of nitric oxide (NO) donors with wound healing properties.

collagens and their subsequent maturation [98]. Collagen is produced by fibroblasts and helps the wound to gain tensile strength during repair [99].

S-Nitrosothiols are another example of NO donors and the most representative member of this class of compounds is *S*-nitrosoglutathione (**50**; Fig. **5**). *S*-Nitrosoglutathione (**50**) is synthesized by living organisms through the *S*-nitrosation of a primary thiol glutathione [100]. *S*-Nitrosothiols are responsible for the reduction of disulfide bridge and proteins transnitrosation [101]. Administration of synthetic *S*-nitrosoglutathione (**50**) in wounded animals increased the collagen content of the scars by 52 and 47% after five and ten days of treatment [95]. The NO donor NOE-aspirin (**51**; Fig. **5**) significantly increased the scar strength in an incision wound model (359 ± 16 g *versus* 271 ± 16 g in non-treated wound). Collagen synthesis was increased by 39.3 % in 10-day-old granulation tissue treated with NOE-aspirin (**51**). NOE-aspirin (**51**) was also shown to promote wound contraction and re-epithelization.

CONCLUDING REMARKS

The mechanism for repairing an injured tissue is not fully understood and the development of efficient therapeutic strategies remains a challenge. Injury of tissues is a very common process. Lesion healing becomes a particular issue for individuals with diabetes where cicatrizing is impaired. Although many studies have examined the effect of plant crude extracts on the wound healing process, only a few studies have reported the effects of individual pure natural products and their derivatives. Many new discoveries are to be made and the most representative examples of natural and synthetic products with wound healing properties are presented in this review. Understanding the mechanism by which these compounds induce wound healing will be valuable for the design of efficient drugs.

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ABBREVIATIONS

GI_{50} = Concentration necessary to inhibit cell growth by 50%

ED_{50} = Effective dose to induce wound healing by 50%

FAD = Flavin adenine dinucleotide

FMN = Flavin mononucleotide

H_4B = Tetrahydrobiopterin

HT_{50} = Time necessary to heal a wound by 50%

LOX = Lysyl oxidase

NADPH = Nicotinamide adenine dinucleotide

NO = Nitric oxide

NOS = Nitric oxide synthase

OPH = Hydroxyproline

PVA = Polyvinyl alcohol

VEGF = Vascular endothelial growth factor

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