

Expert Opinion

1. Introduction
2. Patents on pharmaceutical compositions
3. Patents on naturally occurring phloroglucinol molecules with therapeutic activity
4. Conclusions and future perspective
5. Expert opinion

informa
healthcare

Phloroglucinol compounds of therapeutic interest: global patent and technology status

Inder Pal Singh[†], Jasmeen Sidana, Parikshit Bansal & William J Foley

[†]*National Institute of Pharmaceutical Education and Research (NIPER), Department of Natural Products, Sector-67, S.A.S. Nagar, Punjab 160062, India*

Background: Phloroglucinol compounds, both synthetic as well as natural, have shown a vast array of biological activities. There are a wide range of applications of phloroglucinol compounds in pharmaceuticals, cosmetics, textiles, paints and dyeing industries. Although many of the phloroglucinols have shown promising results in various biological assays, very few have reached clinics. **Objective:** To compile the patented information on various therapeutically active phloroglucinol molecules, so that technologies used in isolation and activity assessment of these compounds could be unearthed and the compiled information be utilized for further development of these molecules. **Methods:** The European Patent Office database (official website: espacenet.com) was searched with a keyword "phloroglucinol". In addition, patents were searched using names of compounds listed in our previous review. **Conclusions:** This class holds potential for development of molecules in various therapeutic areas. There exist a number of patents on preparations that have phloroglucinol compounds as active ingredient(s). Many such preparations have been tested *in vitro* and/or *in vivo* for their efficacy and proven to be active and non-toxic. Commercialization of existing technology on phloroglucinol molecules can yield fruitful results.

Keywords: anticancer, anti-depressant, anti-microbial, anti-protozoal, anti-spasmodic, anti-viral, dermatology, hyperforin, pharmaceutical compositions, phloroglucinol

Expert Opin. Ther. Patents (2009) 19(6):847-866

1. Introduction

Phloroglucinol compounds comprise a family that includes synthetic or semi-synthetic moieties and > 700 naturally occurring compounds. This is an important class of natural products containing 1,3,5-trihydroxy benzene as the basic moiety. A vast array of activities such as anti-inflammatory, anticancer, anti-microbial, anti-allergic, enzyme inhibitory, neuro-regenerative and antioxidant have been exhibited by these compounds. We have earlier published a comprehensive review covering published scientific literature on different classes of naturally occurring phloroglucinol compounds. The present review is complementary to our previous review [1]. To the best of our knowledge, no review has been published previously on the technology status of this class of biologically active molecules or related intellectual property rights issues.

The aim of the present review is to survey various patents obtained on phloroglucinol compounds to evaluate the commercial potential as well as to compile the existing information on global technology status of phloroglucinols. A search for patents with the keyword "phloroglucinol" gave > 600 patents. In the present review, we have covered 94 patents mainly on pharmaceutical applications. We have also included patents on the isolation and purification of phloroglucinol molecules that hold promise in several therapeutic areas. In addition, patents searched using

names of compounds listed in our previous review are also included in this review. The remainder of the patents is on diverse applications such as cosmetics, insecticides, pesticides, adhesives, textiles, resins, paper, cements and polymers and is not included in the present review.

2. Patents on pharmaceutical compositions

Phloroglucinol itself or in combination with other substances as well as derivatives of phloroglucinol have been used as active ingredients in compositions such as anti-spasmodic, anti-viral, anti-malarial, vasodilating and many others. The compositions containing phloroglucinol or its derivatives are discussed in the following sections.

2.1 Anti-spasmodic compositions

Phloroglucinol (1) has anti-spasmodic properties and several anti-spasmodic compositions have been patented that use phloroglucinol or its derivatives as active ingredients. Pharmacological evaluations have demonstrated the safety and therapeutic efficacy of phloroglucinol. Even at a dose as high as 2 g kg⁻¹ intraperitoneally in white mice, no respiratory, cardiac or convulsive toxicity was observed. Intravenous toxicity tests on dogs anaesthetized with chloral also showed no mortality at a dose of 250 mg kg⁻¹. Chronic toxicity studies of phloroglucinol also showed that no risk was involved at doses much higher than active doses and no anti-thyroid action was observed. The anti-spasmodic effect was studied on isolated rat duodena. The duodena from rats fasted for 20 h was collected and maintained using standard Tyrode solution and spasms were induced by 10 mg barium chloride. Addition of 50, 100 and 150 mg of phloroglucinol prevented the spasmogenic effect of barium chloride. In each case, a concentration-dependent anti-spasmodic effect was observed. Similar preventive action of phloroglucinol on barium chloride-induced spasms in the duodenum and ileum of dogs was also observed. However, phloroglucinol did not modify acetylcholine-induced effects on isolated ureters of dogs and guinea-pigs, which were otherwise alleviated by atropine. In clinical tests, cachets containing one part of phloroglucinol and nine parts of glucose showed efficacy as anti-spasmodic agent. It also helped eliminate depression in hepatitis patients and was also effective in treatment of nephritic colic. On the basis of above results, a pharmaceutical composition containing phloroglucinol and different sugars such as glucose, lactose and sucrose has been claimed in cachet, tablet, capsule and injectable forms [2].

Phloroglucinol can also be formulated into effervescent tablets, granules or powders. Apart from phloroglucinol dihydrate, the key ingredients of the formulation include citric acid, sodium bicarbonate and sodium benzoate. The acid-salt combination present in the composition acts as a solid buffer system, which buffers gastric acidity to a pH in range of 3 – 7. The anti-spasmodic activity exhibited by this formulation is appreciably greater than that of oral

lyophilizates and is comparable to that of an intramuscular injection [3,4].

Phloroglucinol ethers also show ureteral and gastrointestinal anti-spasmodic activity as well as hypercholeric activity. The pharmaceutical compositions contain at least one phloroglucinol ether and a pharmaceutically acceptable inert solid carrier in the form of tablets, cachets or capsules. Five ethers, namely 3,5-dihydroxy anisole (2), 3,5-dimethoxy phenol (3), 3,5-dihydroxy phenetole (4), 3,5-diethoxy phenol (5) and 3,5-dipropoxy phenol (6) were prepared and evaluated for toxicity in mice and anti-spasmodic action in rats, dogs and cats. The acute toxicity studies on compound 2 showed DL₅₀ (lethal dose at which 50% of the animals are killed) at 715 mg kg⁻¹ intravenously and 1300 mg kg⁻¹ buccally. Continuous administration at 250 mg (kg⁻¹d⁻¹) for 1 month showed no anomalies. It showed anti-spasmodic effect in isolated ureter in dogs *in vivo* at 10 mg kg⁻¹ intravenously and 150 mg kg⁻¹ intraduodenally. This activity was also observed relative to Oddi's sphincter and ureter. At a dose of 50 mg kg⁻¹ intravenously, 2 produced considerable choleresis in dogs and rats. However, 2 showed no cardiovascular action and did not modify the action of adrenaline or acetyl choline. It also exerted sedative action and potentiated barbiturate sedation. Similarly, DL₅₀ and sub acute toxicity levels of other ethers 3 – 6 and minimum doses at which no anomalies were observed were determined on mice. Compound 3 also showed anti-spasmodic effects relative to intestine, Oddi's sphincter and the uterus and to a lesser degree on ureter (15 mg kg⁻¹ intravenous and 66 mg kg⁻¹ intraduodenally). At the same time, it increased choleresis. It showed no cardiovascular action but decreased the mobility showing its sedative action on CNS. 3,5-Dihydroxy phenetole (4), at a dose of 45 mg kg⁻¹ intravenous, showed anti-spasmodic effect at intestine and Oddi's sphincter. *In vitro*, it diminished spontaneous contractions of isolated dog ureter. Anti-spasmodic effect was also seen in guinea-pigs at a dose of 25 mg kg⁻¹. It did not modify the action of acetyl choline or adrenaline and showed moderate sedative effect. Compound 5 also showed anti-spasmodic effect *in vivo* in guinea-pigs at 10 and 20 mg kg⁻¹ intravenous. It arrested contractions of isolated guinea-pig ureter at doses of 20 and 100 µg ml⁻¹. Compound 6 showed coronary vasodilator effect on isolated rabbit heart at a dose of 100 µg ml⁻¹. It exhibited anti-spasmodic effect on guinea-pig ileus *in situ* at a dose of 250 mg kg⁻¹ intraperitoneally and intraduodenally. In humans, 2 is particularly active in nephritic colic and spasms caused by menstrual disorders. Compound 3 gave excellent results in patients with nephritic or hepatic colic. Compound 4 was effective in treatment of enterocolitis and in migraine conditions [5].

Another composition contains phloroglucinol, an antioxidant such as sodium pyrosulfate, sodium bisulfate, potassium pyrosulfate, water and auxillary components such as sweetener, flavoring agent and pigment [6]. Composition containing

ibuprofen along with phloroglucinol or its dimethyl derivative is also used for anti-spasmodic activity [7].

Anti-spasmodic therapeutic compositions have also been made from 1-(2-chloroethoxy)-3,5-dihydroxy-benzene (7), 3,5-di-(2-chloroethoxy)-phenol (8) [8] and 2-(3,5-dimethoxy-phenoxy)-ethanol (9) and 3-(3,5-dioxy-phenoxy)-1,2-propanediol (10) [9]. These patents also give the synthetic methods along with the acute toxicity and other pharmacological studies on these compounds. Anti-spasmodic effect of 7 was observed on isolated rat duodenum, isolated guinea-pig ureter and ileus.

Another patent describes synthesis and pharmacological studies of nine compounds having anti-spasmodic, choleric, tranquillizing and vasodilating properties. 1-(3',5'-dimethoxy-phenoxy)-2-(*N,N*-diethylamino)-ethane hydrochloride (11), 1-(3',5'-dimethoxy-phenoxy)-2-morpholino-ethane hydrochloride (12), 1-(3',5'-diethoxy-phenoxy)-2-(*N,N*-diethylamino)-ethane hydrochloride (13), 1-(3',5'-diethoxy-phenoxy)-2-morpholino-ethane hydrochloride (14), 1-(3',5'-diethoxy-phenoxy)-2-(*N,N*-diethylamino)-ethane iodomethylate (15), 1-(3',5'-dimethoxy-phenoxy)-2-piperidino-ethane hydrochloride (16), 1-(3',5'-dimethoxy-phenoxy)-2-morpholino-ethane iodomethylate (17), 1-(3',5'-dimethoxy-phenoxy)-2-(*N,N*-diethylamino)-ethane iodomethylate (18) and 1-(3',5'-dimethoxy-phenoxy)-2-piperidino-ethane iodomethylate (19) were synthesized and their anti-spasmodic properties studied *in vitro* using rat duodenum and guinea-pig ureter by observing the relaxation in organs treated with barium chloride and acetyl choline. Compounds 11 and 13 were formulated into injectable ampoules containing 5 – 20 and 6 mg, respectively, of the drug in 5 ml of isotonic sodium chloride solution. Compounds 12 and 14 were formulated into tablets or pastille containing 100 – 200 and 50 mg of active ingredient, respectively. Clinically, these preparations can be used for the treatment of intestinal pains, hepatic colics and enterocolitis [10].

2.2 Anti-viral compositions

Anti-viral compositions containing phloroglucinol or its derivatives, either synthetic or isolated from plants, as active constituents or in combination with other anti-viral components such as AZT, DDC and HIV protease inhibitors are also known. The compounds claimed to have general formula (20) with R_1 – R_6 H, alkyl or acyl chains of varying length, aryl, halogen, nitro, alkylene, alkenylene and so on. References to synthesis of these compounds are given and methods of isolation of compounds from leaves of *Melicope sessiliflora* are also provided. Some specific compounds claimed in this composition are shown in Figure 1. The simple diacyl compounds such as 2,4-dinonanoyl-6-methylphloroglucinol (21), 2,4-di-(4-methylvaleryl)-6-methylphloroglucinol (22), 2,4-diisovaleryl-6-methylphloroglucinol (23), compound 24 and a benzopyran derivative 25 inhibited HIV infectivity in T4⁺ lymphocytes ($IC_{50} < 5.0 \mu\text{g ml}^{-1}$). Compounds 21 – 23, 2,4-divaleryl-6-methyl phloroglucinol (26), 2,4-di-(4-methylvaleryl) phloroglucinol (27), 2,4-dioctanoyl-6-methylphloroglucinol (28) and 2,4-dibutyryl-6-methylphloroglucinol (29) inhibited

HIV syncytia ($IC_{50} < 5.0 \mu\text{g ml}^{-1}$). Two compounds 30 and 31 in which benzopyran is joined to phloroglucinol also showed activity and are claimed in the patent. Other derivatives such as 32 – 35 were inactive. Pro-drugs of phloroglucinol or its derivatives, for example, phloroglucinol or derivatives of phloroglucinol bound to other chemical groups that may enhance stability or reduce toxicity are also included in this patent. The compounds can be administered parenterally, orally, rectally, buccally or transdermally and with a pharmaceutically acceptable carrier or diluent. The compounds can be administered in solid or liquid forms [11].

There are two Japanese patents on anti-herpes virus activity of phloroglucinol compounds. Diacyl phloroglucinol compounds such as 2,4-diacetylphloroglucinol (36) prepared by reacting phloroglucinol with corresponding acid in presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ can be used in patients infected with herpes virus. Administration of phloroglucinol compounds can be either oral or by inhalation [12,13] (see Figure 1 for structures 1 – 36).

2.3 Anti-protozoal and anti-microbial compositions

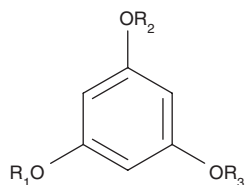
Compositions have been described for natural products with anti-malarial activity. These include aspidinol (37), methylene bis-aspidinol (38), desaspidin (39), aspidin (40), flavaspidic acid (41) and albaspidin (42). These compounds have significant activity, especially methylene bis-aspidinol, which showed an IC_{50} value of 325 ng ml^{-1} against W_2 strain and 574 ng ml^{-1} against D_6 strain of *Plasmodium falciparum*. Preparations containing 800 ng ml^{-1} of methylene bis-aspidinol, 1600 ng ml^{-1} of desaspidin and 1800 ng ml^{-1} of aspidin have been formulated for use as potential anti-malarial medications [14].

Caespitate (43), a phloroglucinol compound isolated from dried aerial parts of *Helichrysum caespititium*, demonstrated significant anti-bacterial and anti-tubercular activity. Caespitate showed an MIC of 0.5 – 5 $\mu\text{g ml}^{-1}$ against most of the Gram-positive bacteria and similar activity against common fungal species. It showed a minimum inhibitory concentration of 0.1 mg ml^{-1} against drug resistant strains of *M. tuberculosis*. It has been formulated into compositions of pharmaceutical use for the treatment of infections caused by pathogenic bacteria, mycobacteria and fungi in humans [15].

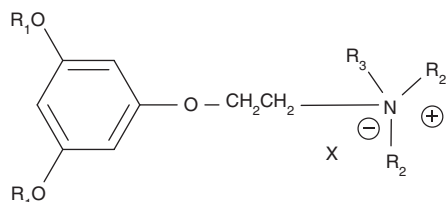
Various plant species contain phloroglucinol compounds as components of the essential oils present in leaves. *Leptospermum scoparium* contains leptospermone (44), isoleptospermone (45) and flavesone (46) apart from the terpenes present in the essential oil. This oil as whole or in diluted form inhibited the growth of both Gram-negative and Gram-positive bacteria and can be used as an anti-microbial medication. Its mixture with essential oil of *Melaleuca alternifolia* in different proportions has been patented for use as a topical preparation [16].

Another Japanese patent describes the use of naturally occurring phloroglucinol compounds as anti-bacterial agents. Compounds of phlorotannin class such as eckol (47), phlorofucofuroeckol A (48), dieckol (49) and 8,8'-bieckol (50) are used as anti-bacterial agents alone or in combinations [17].

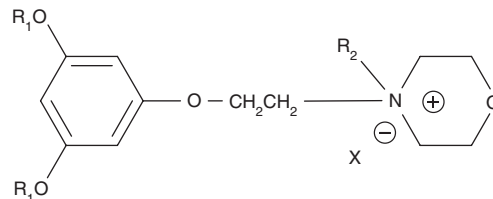
Phloroglucinol compounds of therapeutic interest: global patent and technology status



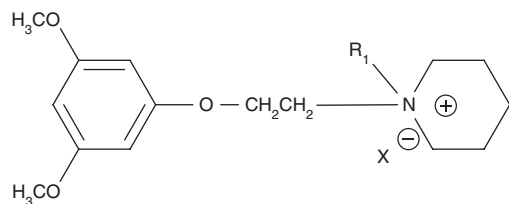
- 1: $R_1 = R_2 = R_3 = H$
 2: $R_1 = CH_3; R_2 = R_3 = H$
 3: $R_1 = R_2 = CH_3; R_3 = H$
 4: $R_1 = CH_2CH_3; R_2 = R_3 = H$
 5: $R_1 = R_2 = C_2H_5; R_3 = H$
 6: $R_1 = R_2 = C_3H_7; R_3 = H$
 7: $R_1 = R_2 = H; R_3 = CH_2CH_2Cl$
 8: $R_1 = H; R_2 = R_3 = CH_2CH_2Cl$
 9: $R_1 = R_2 = CH_3; R_3 = CH_2CH_2OH$
 10: $R_1 = R_2 = CH_2CH_3;$
 $R_3 = CH_2CH(OH)CH_2OH$



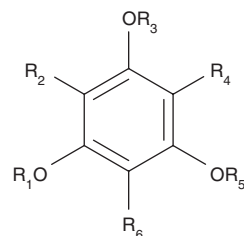
- 11: $R_1 = CH_3; R_2 = CH_2CH_3; R_3 = H; X = Cl$
 13: $R_1 = R_2 = CH_2CH_3; R_3 = H; X = Cl$
 15: $R_1 = R_2 = CH_2CH_3; R_3 = CH_3; X = I$
 18: $R_1 = R_3 = CH_3; R_2 = CH_2CH_3; X = I$



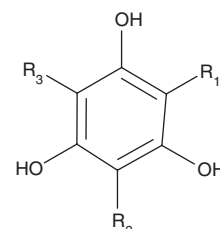
- 12: $R_1 = CH_3; R_2 = H; X = Cl$
 14: $R_1 = CH_2CH_3; R_2 = H; X = Cl$
 17: $R_1 = R_2 = CH_3; X = I$



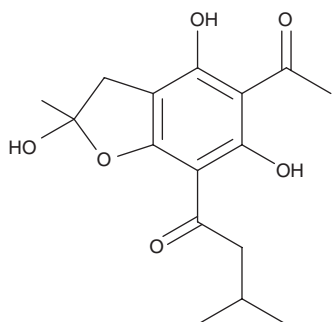
- 16: $R_1 = H; X = Cl$
 19: $R_1 = CH_3; X = I$



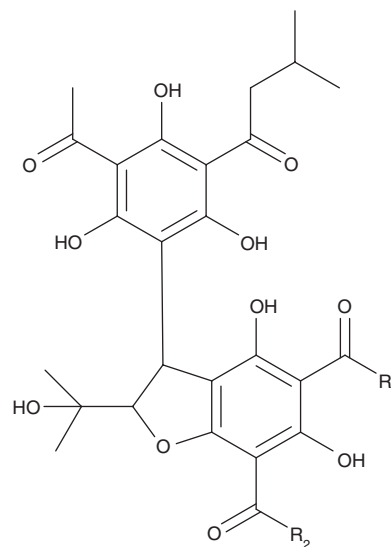
20



- 21: $R_1 = R_2 = \text{nonanoyl}; R_3 = \text{methyl}$
 22: $R_1 = R_2 = 4\text{-(methylvaleryl)}; R_3 = \text{methyl}$
 23: $R_1 = R_2 = \text{isovaleryl}; R_3 = \text{methyl}$
 26: $R_1 = R_2 = \text{valeryl}; R_3 = \text{methyl}$
 27: $R_1 = R_2 = 4\text{-(methylvaleryl)}$
 28: $R_1 = R_2 = \text{octanoyl}; R_3 = \text{methyl}$
 29: $R_1 = R_2 = \text{butyryl}; R_3 = \text{methyl}$
 36: $R_1 = R_2 = \text{acetyl}; R_3 = H$



25



- 30: $R_1 = CH_3; R_2 = CH_2CH(CH_3)_2$
 31: $R_1 = CH_2CH(CH_3)_2; R_2 = CH_3$

Figure 1. Phloroglucinol compounds used in anti-spasmodic and anti-viral compositions.

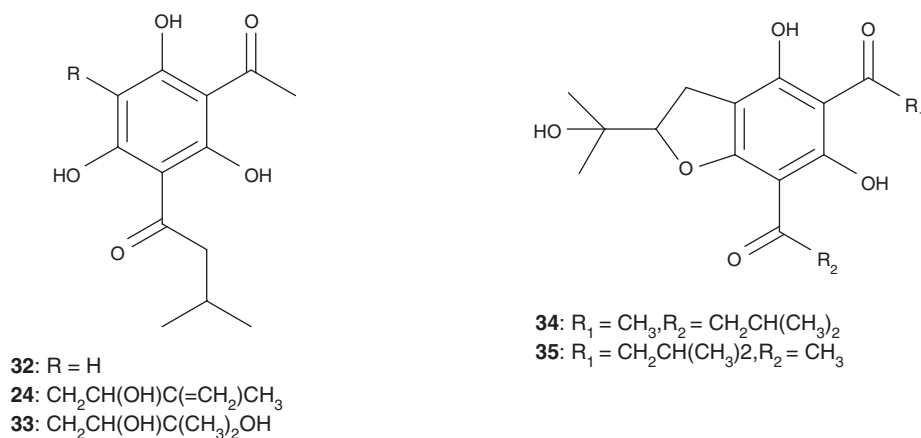


Figure 1. Phloroglucinol compounds used in anti-spasmodic and anti-viral compositions (continued).

These compounds were isolated from *Ecklonia stolonifera* and exhibited urease inhibitory activity. These can be used in food, medicinal and chemical field for preventing ageing and oxidation [18,19]. The same compounds, either alone or in combination, have also been patented for use as antiviral agents [20].

Panduratin (51) and its derivatives showed high anti-bacterial activity against *Streptococcus mutans* (dental caries inducing bacteria) and *Porphyromonas gingivalis* (periodontal disease-inducing bacteria). Panduratin derivatives are thermo-stable and, therefore, could be extracted in high yields in reduced extraction times from *Kaempferia pandurata*. The oral composition contains isopanduratin (52) as an active ingredient. It can be formulated as an anti-bacterial agent in the form of toothpaste, mouth wash, gum, candy, jelly or chocolates [21].

Compounds such as humulone (53), cohumulone (54) and adhumulone (55) have also been used in compositions for infectious diseases. The composition has strong activity against methicillin-resistant *Staphylococcus aureus* [22]. Formulation is also reported using these compounds for antioxidant action and for treating diseases such as rheumatoid arthritis and lesions such as scald and acne. This antioxidant composition may also be used to prevent degradation of foods and drinks [23]. Mallophenone (56) or its salts can also be used in formulations for preventing or treating diseases such as rheumatism, acne and dermal stains [24].

2.4 Anthelmintic compositions

Diacyl phloroglucinol derivatives show significant anthelmintic activity. Several C-acylated phloroglucinol compounds were synthesized; 2,4-dibutyrylphloroglucinol, 2,4-diisobutyrylphloroglucinol and 2,4-divalerylphloroglucinol were more active than the others. These compounds are capable of forming non-toxic salts with pharmaceutically acceptable strong inorganic and organic bases and, therefore, can be used in compositions. 2,4-Di-n-valerylphloroglucinol (57) was formulated into tablets containing 200 mg as active ingredient. These compounds

exhibited high specificity in the host against various helminthic infections of the intestinal tract. These tablets can be administered to humans 1 – 6 times daily depending on the extent of helminthic infections [25] (See Figure 2 for structures 37 – 57).

2.5 Dermatological compositions

Formulation containing 5-methoxypsoralen (58) has been patented for treating psoriasis and other skin disorders. Method for preparation of 5-methoxypsoralen from phloroglucinol is also given. In general, furanocoumarins, especially psoralens show photodynamic activity after topical or oral administration followed by exposure to UV rays. It is also known that therapeutic activity of psoralens is directly proportional to their phototoxicity. Psoriasis is treated by oral administration of 8-methoxypsoralen (59) followed by irradiation at 360 nm twice a week. The results are extensive bleaching and disappearance of psoriatic plaques. Compound 58 is less phototoxic and, therefore, less therapeutically active than 59, but it has higher therapeutic index (therapeutic activity/acute toxicity) and can be administered at much higher doses than 59. Acute toxicity studies were carried out on male mice and rats and 59 was found to be 17 times more toxic than 58. Long-term general toxicity studies (gastric administration for 42 consecutive days) in rabbits showed that the drug was well tolerated. Similar conclusions were drawn after application of oily preparation of the drug on skin. The pharmacological activity of 58 was studied in patients suffering from psoriasis by administering 58 orally or locally and followed 2 h later by irradiation at 360 nm. Several case studies are reported demonstrating up to 95% bleaching of psoriatic plaques after 20 – 30 treatment sessions. The drug can be given in different forms such as tablets, oil, aqueous emulsion or ointment. In several cases, some psoriatic spots persisted after oral treatment, which could be treated by local application. The drug was well tolerated in patients and there was no recurrence of psoriasis for 6 months [26,27].

Phloroglucinol compounds of therapeutic interest: global patent and technology status

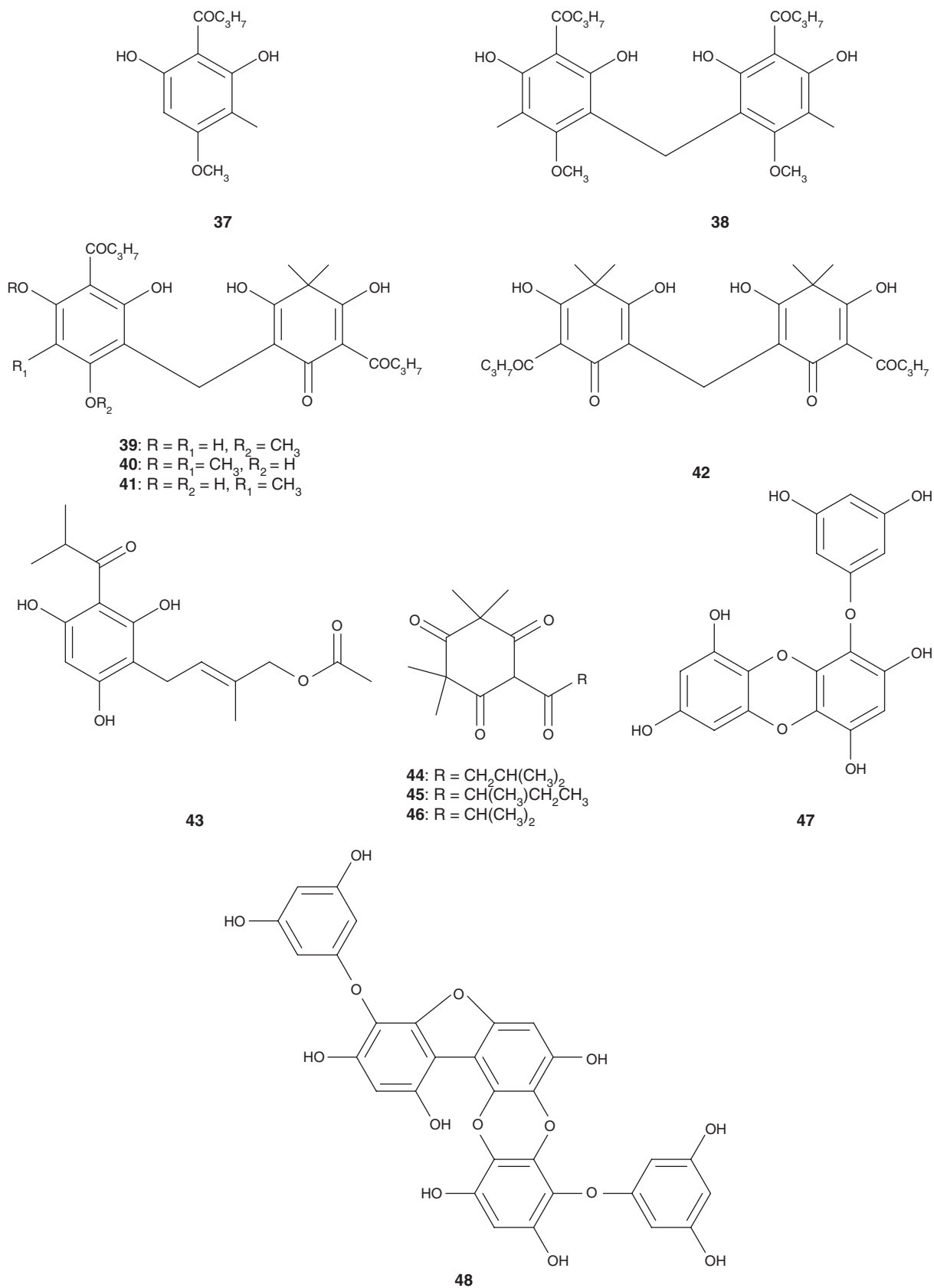


Figure 2. Phloroglucinol compounds used in anti-microbial and anti-protozoal compositions.

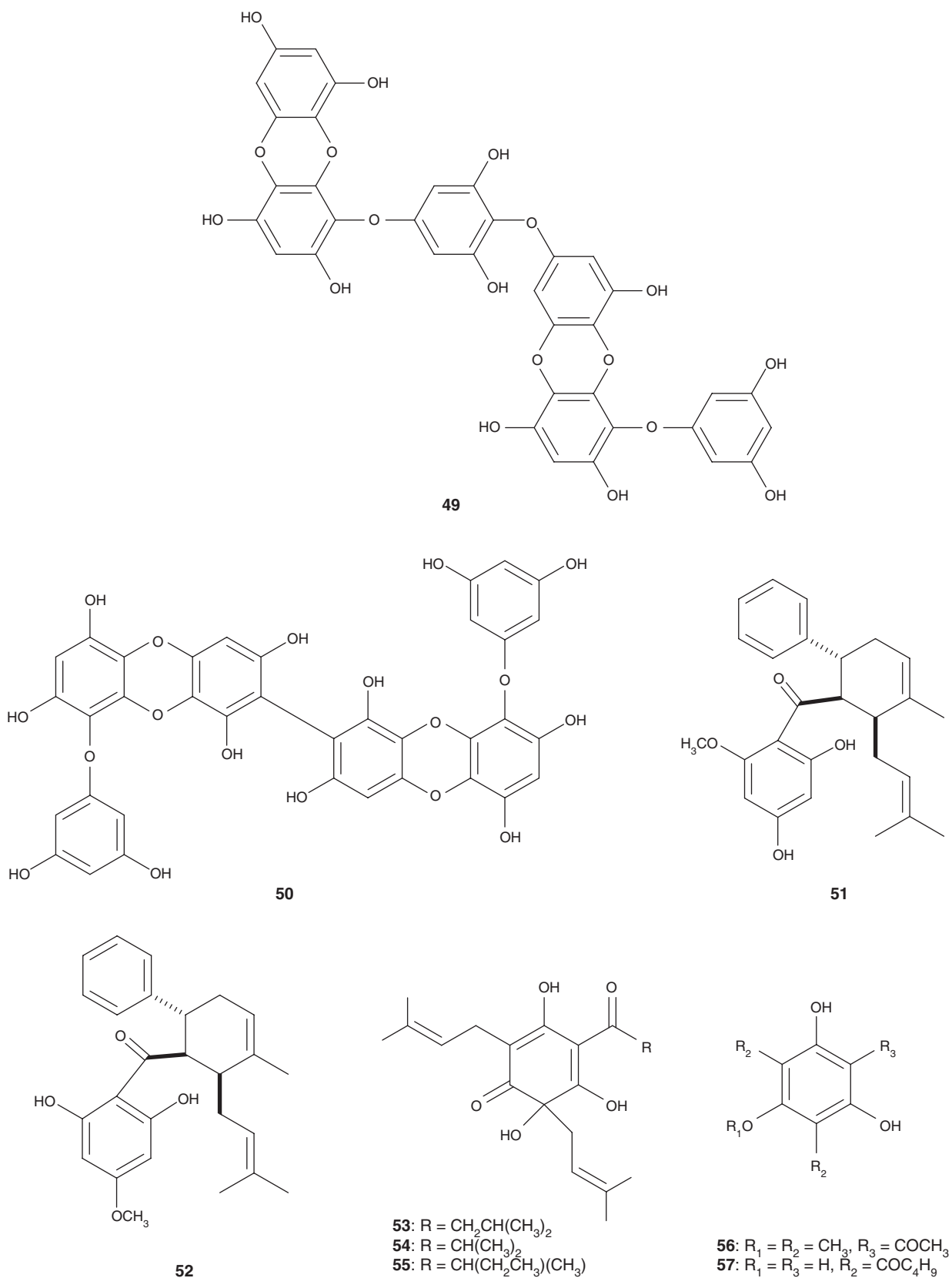


Figure 2. Phloroglucinol compounds used in anti-microbial and anti-protozoal compositions (continued).

A chlorophyll free extract of the aerial parts of myrtle (*Myrtus communis* L.) containing non-polar constituents is claimed for treating psoriasis and keratinization disorders. The extract showed anti-bacterial, anti-inflammatory and anti-proliferative properties. The extract is prepared in such a way as to eliminate essential oils and tannins that were earlier supposed to be active constituents. Apart from myrtucommulone A (60) and myrtucommulone B (61), other constituents of the extract are triterpenoids, steroids and plant pigments. The extract can be formulated as lotion, cream, shampoo or spray [28].

Amino ketone derivatives of phloroglucinol especially buflomedil (62) are used in Raynaud's disease, acrocyanosis and other alopecial conditions of skin. These compounds when administered in the form of liposomal aqueous micro-dispersion increase the volume and flow rate of blood to the skin. Topical preparations in which buflomedil hydrochloride is complexed with phospholipids such as lecithin or cephalin are used in capillary vascularization of hairy and unsightly skin [29].

Many natural and synthetic phloroglucinol derivatives are used in pharmaceutical preparations for epidermal renewal and proliferation of keratinocytes. Apart from phloroglucinol itself, such preparations may contain 5-methoxy-1,3-benzenediol or flamenol (2), 3,5-dimethoxyphenol or taxicatigenin (3), aspidinol (37), desaspidin (39), leptospermone (44), humulone (53), 1,3,5-trimethoxybenzene (63), 1,3,5-triethoxybenzene (64), 1,3,5-triphenoxybenzene (65) and tasmanone (66). The percentage of active phloroglucinol moiety alone or in combination varies from 0.001 – 5% w/w. Such preparations can be used for combating chronobiological or photo-induced ageing of skin, for cicatrization of human skin and for alleviating the undesirable effects of menopause on skin [30].

Another patent describes a formulation prepared from *Mallotus japonicus* extract. Skin lotion, nutrient cream, astringent lotion, cream or essence is prepared using whole dried extract of *M. japonicus*. These preparations are claimed to have elastase inhibitory and collagen biosynthesis stimulatory activity and can be used for anti-wrinkle effects [31]. Pure extract of *Acacia meamsii*, which contains phloroglucinol and other polyphenols, is also used for treating skin disorders caused by sun exposure, microbial infestation, burns and so on. The preparation is said to regenerate skin layers by stimulating blood circulation and is intended for use in cells infected with AIDS virus and cancer [32].

2.6 Other pharmaceutical preparations

Many of the conventional and recent dosage forms can be designed using amino ketone derivatives of phloroglucinol and their salts, as active ingredients for the treatment of entrapment and compression of nerves. Oral dosage forms such as tablets, capsules, caplets, solutions, suspensions and syrups and more recent ones such as encapsulated beads, granules, pellets and transdermal patches were manufactured

using these derivatives. These preparations were found to be active against nerve damage caused by surgery or physical injury such as Kiloh Nevin syndrome, pseudo-carpal tunnel syndrome, Tardy Ulnar palsy and Guyon's Canal syndrome. A group of compounds consisting of 4-(pyrrolidinyl)-1-(2,4,6-trimethoxyphenyl)-1-butanone, also named as buflomedil (62), (2,4,6-trimethoxyphenyl) (3-piperidinopropyl) ketone (67), (2,4,6-triethoxyphenyl) (3-diethylaminopropyl) ketone (68) and (2,4,6-triethoxyphenyl) (3-pyrrolidinopropyl) ketone (69) were formulated into tablets containing 600 mg of active ingredient and 450 mg of magnesium oxide as pharmaceutically acceptable carrier along with other suitable additives. These compounds can be administered along with calcium channel blockers such as felodipine, amlodipine, nifedipine and α -adrenoreceptor antagonists cetiedil and ergotamine [33,34].

Acylated and prenylated derivatives of phloroglucinol have also been used as active ingredients of pharmaceutical compositions to treat osteoporosis by promoting bone formation or inhibiting bone resorption. These derivatives naturally found in *Humulus lupulus* L. (brewing hops) have a structure similar to that of prostaglandin E₂, and so are capable of acting on osteoblasts to inhibit bone resorption. Five and six membered α -acids such as humulone (53), cohumulone (54) and adhumulone (55) or iso- α -acids such as isohumulone (70), isocohumulone (71) and isoadhumulone (72) were found to inhibit bone resorption at a concentration as low as 1×10^{-9} M in the pit formation assay. Solid oral preparations such as ordinary tablets, capsules, granules, powders and enteric-coated tablets have been formulated using one or more of the above mentioned active ingredients. Injectable powders have also been formulated and can be reconstituted into a suitable vehicle before use. A daily dose of 0.1 – 2.0 g of individual component or in combination is recommended [35].

Several synthetic phloroglucinol compounds possess vasodilating properties; these include (2,4,6-trihydroxyphenyl)-(3-pyrrolidinopropyl)-ketone hydrochloride (73), (2,4-dimethoxy-6-hydroxyphenyl)-(3-pyrrolidinopropyl)-ketone hydrochloride (74), (2,4,6-trihydroxyphenyl)-(3-diethylaminopropyl)-ketone hydrochloride (75), (2,6-dihydroxy-4-methoxyphenyl)-(3-pyrrolidinopropyl)-ketone hydrochloride (76) and (2,6-dimethoxy-4-hydroxyphenyl)-(3-pyrrolidinopropyl)-ketone hydrochloride (77). Vasodilatory activity of these compounds was studied in anaesthetized dogs by intravenous or intraduodenal administration. Clinically, these compounds are useful in the treatment of Raynaud's disease. A dose of 100 – 200 mg two to three times a day can be given in the form of tablets or gelules. Injectable ampoules have also been formulated containing 50 – 100 mg of the active ingredients and are used in cerebral vasodilation [36].

Tricyclic dibenzopyran-1-ols (78 – 83) containing a phloroglucinol moiety and synthesized from phloroglucinol have shown significant anti-hypertensive activity that was determined in male SH rats by measuring the changes in blood pressure. Oral or parenteral preparations can be made from these compounds using conventional methods and

can be used to lower the blood pressure in humans [37]. Derivatives of substituted phloroglucinol with an amide grouping (84 – 86) show analgesic and anti-inflammatory properties. Several related compounds were synthesized and evaluated for toxicity, analgesic and anti-inflammatory activity in mice. The test for analgesic activity was based on a study of the pain syndrome after injection of acetic acid and observation of the number of contractions. Many of the compounds showed analgesic action in animals and anti-inflammatory action in the Domenjoz's test. In the clinical trials, 11 out of 15 patients suffering from cervicalgias, dorsalgias and neuralgias of head and trunk showed excellent tolerance and positive results. Pharmaceutical composition can be administered by oral, parenteral or rectal route and can be in form of coated tablets, cachets, capsules, injectable ampoules or drinkable suspensions [38,39].

Phloroglucinol is also used in antitumor formulations. The preparations contain eriocitrin (87) and/or eriodictyol (88), 3,4-dihydroxycinnamic acid (89) and phloroglucinol (1) as active ingredients and serve as apoptosis inducing agents [40]. Phloroglucinol derivatives also show leukotriene and thromboxane A2 antagonistic activity. Single dose compositions have been prepared using 1 – 1000 mg of active ingredient and are used as anti-allergic medications [41]. Another phloroglucinol derivative, that is, 2,6-diisobutyryl-4,4-diethylcyclohexane-1,3,5-trione (90) has been used in the manufacture of anti-allergic medications [42].

Phloroglucinol based compounds are also used in the formulations to improve sexual dysfunction. Around 0.1 – 50% of seaweed extract of brown algae, *Sargassum fulvellum*, green laver, *Ecklonia cava*, *Hizikia fusiforme*, *Rhei rhizoma*, laver and *Gracilaria verrucosa* are used along with *Zizyphi fructus* extract, ginseng and grape juice. It is claimed that such preparations cause longer lasting erections and more powerful ejaculations in patients with erectile dysfunction [43].

Phloroglucinol β -D-glucoside, phlorin (91) is used in the photodynamic therapy of age related macular degeneration of eye. Formulations containing phlorin (91), chlorin (92), purpurin (93) and green porphyrin (94) are used in the diagnosis and therapy of unwanted neovascularity in the eye [44] (see Figure 3 for structures 58 – 96).

2.7 Patents on compositions containing hyperforin as active ingredient

More than 40 patents were identified using hyperforin (95), another important phloroglucinol compound, as a keyword. These patents explain the extraction procedures, methods of synthesis, stability characteristics and formulations containing hyperforin or its derivatives. Hyperforin, its semi-synthetic derivatives and the extracts obtained from natural sources are used in the treatment and prophylaxis of dementia diseases.

2.7.1 Anti-depressant compositions

Orally active compositions have been prepared from St. John's Wort extracts. Supercritical extracts made using compressed

carbon dioxide as solvent contain 20 – 50% of hyperforin along with other ingredients such as terpenes, phloroglucinols, phytosterols, fatty acids and waxes. Tablets and capsules have been formulated using 270 – 650 mg of this extract and can be administered in single or divided doses. Oral liquids such as suspensions are also prepared using the extract. Suitable emulsifiers as lecithin and thickening agents such as cellulose derivatives are used. The said compositions are claimed to promote emotional well being in humans and animals [45].

Common plant constituents such as rutin, amentoflavone and essential oils present in *Hypericum* are responsible for the stability of hyperforin in the plant and whole plant extracts. The *Hypericum* biomass can be extracted using supercritical, critical or near critical fluids such as carbon dioxide, nitrous oxide, ethylene, ethane and propane. Methanol, ethanol or propanol can be used as modifiers. The fractions obtained are biologically standardized by measuring the inhibition of serotonin re-uptake and are formulated into hard tablets or soft gelatin capsules. Stability of the fractions can be increased by using antioxidants such as tocopherols and bioavailability can be increased by emulsifiers such as lecithin and various vegetable oils such as olive oil [46].

Another Chinese patent claims the use of *Hypericum* extract in formulation of oral medicaments for the treatment of depression. The medicine named "Jinyukang" contains 2 – 46% of hyperforin along with hypericin, pseudohypericin and flavanoids [47,48].

Saturated derivatives of hyperforin and adhyperforin (96) can be obtained by reduction of hexane extract of *Hypericum*. These derivatives can be coupled with anti-cholinesterase inhibiting alkaloids such as galantamine, physostigmine, vincamine, memantine and tacrine using simple procedures such as dissolution of both ingredients in a cosolvent. The products obtained were formulated into hard gelatin capsules and transdermal preparations for the treatment of depression and Alzheimer's disease [49].

Another patent describes the use of hyperforin–fatty acid conjugates in the treatment of depression and other cognitive disorders. ω -3-Fatty acids show anti-depressant activity by virtue of their effects on lipid membranes and enhancement of serotonergic channels. Apart from the various biological functions, fatty acids suppress the formation of superoxides, which degrade hyperforin and related compounds and also increase the transportation of neurotransmitters across the blood–brain barrier. For the preparation of dosage forms, commercially available hyperforin extract was suspended in the solution of fatty acid. Various polyunsaturated fatty acids with carbon chain length of 12 – 24 were used in formulations that can be administered through oral, intranasal, sublingual, intramuscular and other parenteral routes [50].

Derivatives of hyperforin and adhyperforin prepared by reduction were found to be active as anti-depressant agents in rats. They also have anti-Alzheimer's potential as they activate α -secretase. The enzyme is involved in increased

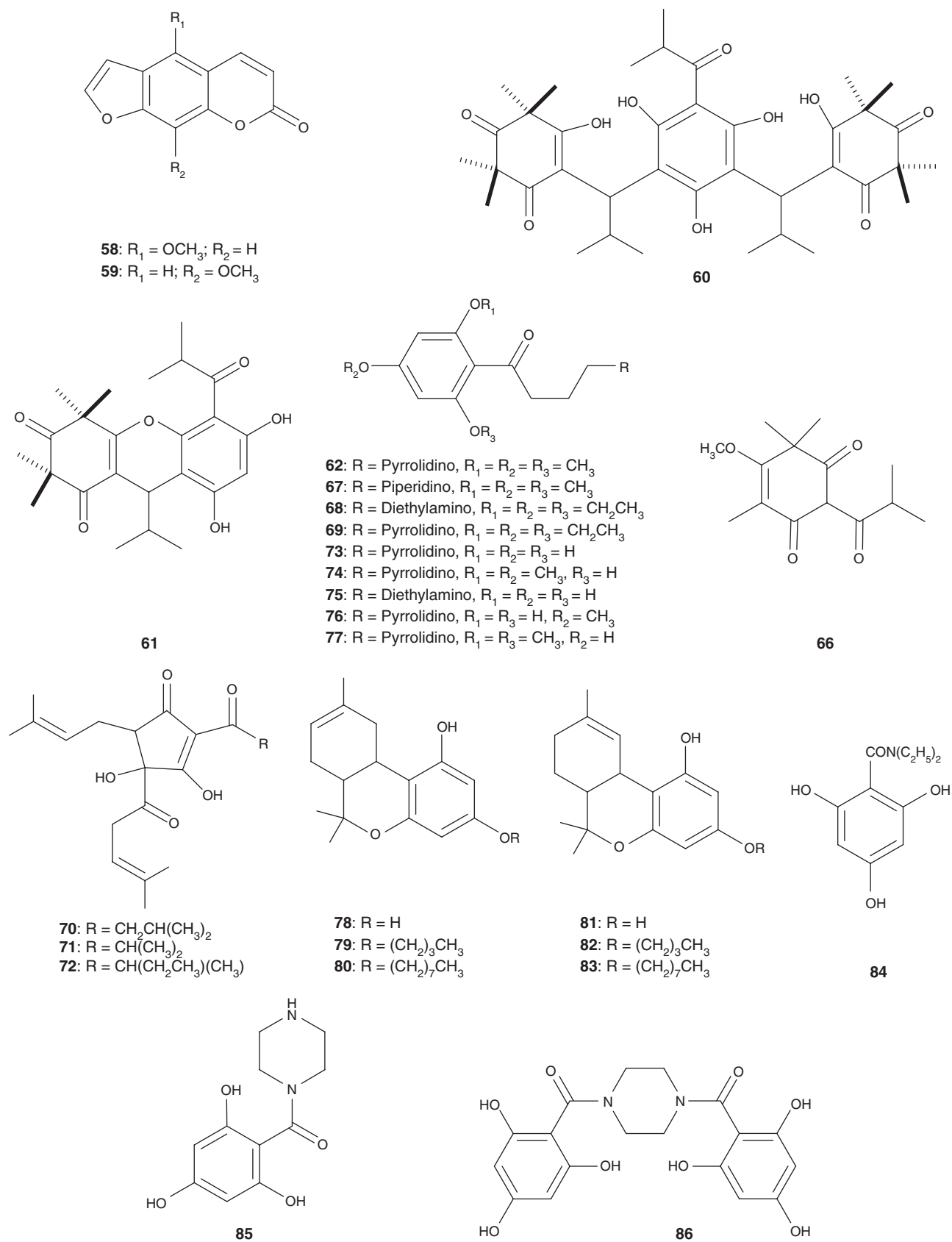


Figure 3. Compounds used in various other pharmaceutical preparations.

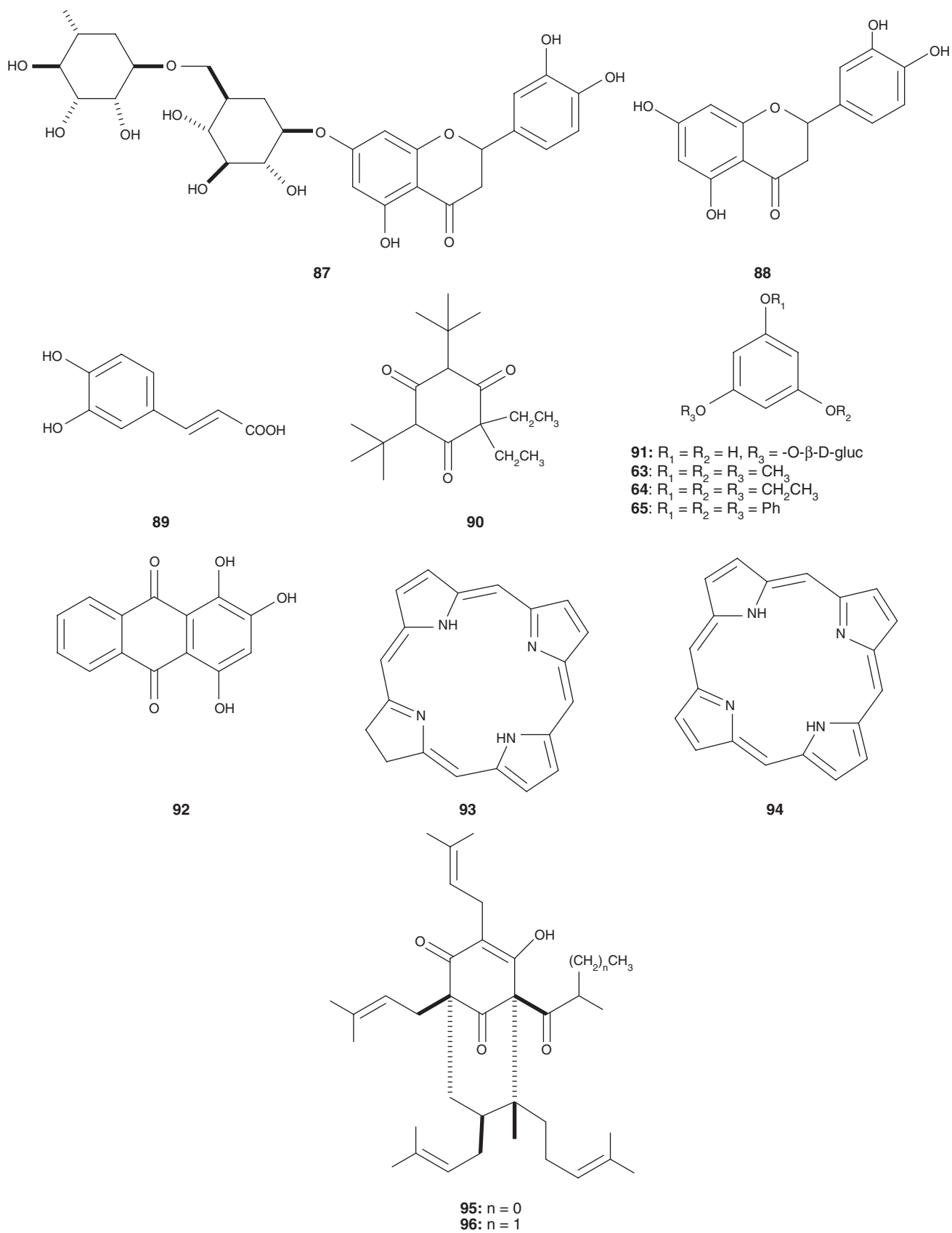


Figure 3. Compounds used in various other pharmaceutical preparations (continued).

production of Alzheimer precursor proteins, which have no pathogenic activity [51].

Derivatives of hyperforin with halogen at carbon number 8 and the derivatives made by reduction of isoprene side chains are more active than hyperforin itself as anti-depressants, anxiolytics and anti-neurodegenerative compounds. Hard and soft gelatin capsules, tablets, suppositories and controlled release formulations have been prepared by usual methods using 8-chloro and 8-bromo salts of hyperforin and adhyperforin [52].

A similar patent describes the semi-synthesis of hyperforin derivatives and their formulation to soft gelatin capsules and controlled release dosage forms. Salts of hyperforin made by replacing the H of hydroxyl group with aliphatic, cycloaliphatic or aromatic acyl residues or glycidic residues in which one or more hydroxyl groups are alkylated or acylated were claimed to be more stable than hyperforin [53].

The stability of hyperforin can be increased by addition of certain additives to the extracts. The stabilizer can be an organic thiol such as cysteine or glutathione, ascorbic acid or its derivatives or fatty acid esters. The stabilized extracts so obtained can be used in the formulation of pharmaceutical preparations such as film coated tablets and soft gelatin capsules and used for treatment of depression and psychovegetative disorders [54-58]. Anti-depressant medicaments have also been prepared from hyperforin acyl and glycoside derivatives [59]. Hyperforin phenolic acid derivatives formulated as injections are used in treatment of depression and hepatitis A and B [60].

2.7.2 Anticancer compositions

Hyperforin formulations have also been used or treating various types of cancer and precancerous forms. A topical ointment or cream can be manufactured from aqueous, ethanol or carbon dioxide extract of *Hypericum* containing not < 200 $\mu\text{g ml}^{-1}$ of hyperforin. According to this patent, an ointment or a cream should contain NLT 15 and 10% of active extract, respectively. Topical preparations are useful in eczema, dermatitis, psoriasis and geriatric skin. Parenteral preparations containing hyperforin have also been formulated using suitable vehicles that can be injected epicutaneously or directly in the tumors so that the concentration of hyperforin in plasma is not less than 50 $\mu\text{g ml}^{-1}$. Hyperforin showed anti-proliferative effects and induced apoptosis in tumor cell lines HT144, A431, Jurkat, 1F6 and MT450 [61]. A similar patent describes the preparation of topical creams and ointments from St. John's Wort extract containing 1 – 15 mg ml^{-1} of hyperforin. Topical preparations of hyperforin along with hypericin are also used in treatment of inflammatory skin diseases, skin aging and bacterial skin infections [62-64].

Constituents of *Hypericum* also find use in photodynamic diagnosis and therapy. Pharmaceutical preparations for combined photodynamic diagnosis and therapy have been manufactured using hypericin and hyperforin along with the diagnostic unit for diagnosis and therapy of mouth and brain malignancies [65].

2.7.3 Other therapeutic preparations of hyperforin

Capsules formulated using dried extract of *Hypericum* containing about 2% hyperforin are useful in the treatment of metabolic syndrome [66]. Another composition containing hyperforin-rich extract is obtained by hypercritical high-pressure extraction of St. John's Wort with carbonic acid. Up to 39% of hyperforin can be extracted by this method and is formulated into various pharmaceutical compositions along with *Usnea barbata* extract. These compositions have been used in the treatment of dermatitis, aging, acne and other skin infections [67]. Another extract stable with respect to color and hyperforin content has been formulated into gels for treating skin disorders such as acne, dermatitis, herpes zoster and burns [68].

Hyperforin can also be used in the treatment of disease in which pathological angiogenesis occurs. Hyperforin and adhyperforin can be used for treating angioproliferative retinopathy, endometriosis, skin diseases and disorders of bones and joints [69]. Another patent claims the use of St. John's Wort extracts in prophylaxis and therapy of anaphylactic shock and osteoporosis [70].

Hypericin and hyperforin, along with ginkgolides and flavonoids, are used as antioxidants and anti-proliferative agents. It is a well-known fact that iron in its ferrous form is involved in lipid peroxidation leading to oxidative stress. Oxidative stress in turn can result in Parkinsonism, Alzheimer's, Crohn's disease, ulcerative colitis and many forms of cancer. Ginkgolides, flavonoids and hypericum metabolites are able to chelate Fe^{2+} and their ability to do so can be assessed by ferrous chelation assay. These herbal extracts and pure compounds can inhibit the formation of iron ferrozine complex and are useful in treatment of iron overload and conditions arising owing to iron overload [71].

Hyperforin obtained from natural sources is conjugated to form salts with amine drugs such as dicyclohexylamine, imipramine, desipramine, memantin and deprenyl that are themselves used in treatment of Alzheimer's disease. These salts were more stable than hyperforin itself when monitored by HPLC for a period of 12 weeks. PKC and α -secretase activity of these salts was comparable to that of hyperforin itself. About 35 conjugates were prepared and formulated into oral, parenteral or rectal medications. These can be administered in doses amounting in total to 0.05 – 5 mg kg^{-1} body weight per 24 h period, if necessary in the form of 1 – 5 unit doses [72].

St. John's Wort extract rich in hyperforin is also reported to enhance male sexual performance as well as relieving bladder irritation and reducing urinary urgency. The hyperforin rich extract can be administered in various dosage forms; sublingual administration is preferred as it allows hyperforin to escape the first pass metabolism and gives rapid rise in serum levels of drug. It induces peak levels of hyperforin in 1 h. The pharmacological response of hyperforin is similar to anti-depressant drugs such as imipramine or fluoxetine through inhibition of serotonin reuptake [73].

2.8 Miscellaneous compositions of medicinal importance

Apart from pure medical applications, phloroglucinol and its derivatives also find use as artificial sweeteners, plasma substitutes and in vitamin compositions. Derivatives of phloropropiophenone were synthesized from substituted aromatic aldehyde and phloroglucinol in five steps. 3-Arylphloropropiophenone derivatives such as hesperetin dihydrochalcone and 3-(*m*-hydroxyphenyl) phloropropiophenone (97) were synthesized in yields varying from 35 to 45% and formulated into an artificial sweetener composition. The key step of whole formulation process was solubilization of the active ingredient, that is, phloropropiophenone derivative. These derivatives are not substantially water soluble, but exhibit sweetener properties when properly dissolved. Various ingestible polar aldehydes and ketones such as isopulegone and acetophenones, variety of alcohols, acids, esters and their mixtures have been used as solvents for phloropropiophenone. Another important category of solvents include natural oils such as sweet birch oil, spearmint, oil of winter green, anise oil and pine oil. They also act as flavors in the composition enhancing the organoleptic appeal [74]. These artificial sweeteners can be used in pharmaceutical formulations.

Polyvalent phenols such as phloroglucinol and their substituted derivatives also find applications in preparation of compositions such as blood and plasma substitutes. Conventionally, plasma substitutes are formed by partial degradation of gelatin previously treated with formaldehyde that serves as a crosslinking agent, to obtain favorable colloido-chemical and physiological properties. Phloroglucinol, resorcinol and methyl resorcinol speed up this crosslinking process [75].

Polyvalent phenols are also used in purification of vitamins. Phloroglucinol, orcinol and hydroquinone form a stable complex with cyanocobalamin having an empirical formula $C_{66}H_{88}N_{14}O_{14}PCo(R)_n(H_2O)_x$, where R is polyvalent phenol and *n* varies from 4 to 11. Here, phenol is added to an aqueous solution of vitamin, resulting in the formation of insoluble complex. Phenol-vitamin crystals are recovered and solubilized in water. Pure vitamin B₁₂ can be obtained by adding a water miscible non-solvent to the solution [76].

3. Patents on naturally occurring phloroglucinol molecules with therapeutic activity

There are a few patents describing the extraction and isolation procedures of naturally occurring phloroglucinol compounds, preparation of their semi-synthetic derivatives, formulations containing these and pharmacological assays for the assessment of activity. We discuss in this section selected representative and novel isolation procedures of naturally occurring phloroglucinol compounds and their activity profiles.

Anti-bacterial and anti-tubercular caespitate (43) was isolated from aerial parts of *H. caespitium*. Maceration with acetone for 5 min yielded crude extract that was fractionated over silica gel column using chloroform as eluent. Fractions collected

were evaluated for anti-bacterial activity by thin layer chromatography on silica gel impregnated with *S. aureus*. Active fractions were purified by reverse phase HPLC and caespitate was detected at 206 nm. Anti-bacterial and anti-fungal assays of caespitate showed that it was active against most of the Gram-positive bacteria and inactive against Gram-negative bacteria. *Aspergillus niger* and *Aspergillus flavus* two fungi infectious to human beings were inhibited by caespitate at a minimum inhibitory concentration of 1.0 µg ml⁻¹ [15].

Three new compounds (98 – 100) with fungicidal potential were isolated from *Helichrysum decumbens*. The crude extract obtained after extraction of fresh plant material with chloroform was fractionated into flavonoid and phloroglucinol fractions by flash chromatography over kieselgel using hexane-ethyl acetate gradient. The phloroglucinol fraction was subjected to chromatography over sephadex LH20 using methanol as eluent. The fractions of interest were analyzed by HPLC using methanol-phosphate buffer as mobile phase. Compounds 98 – 100 were separated by preparative HPLC and assayed for their fungicidal potential. Compound 100 showed activity against *Mucor* and *Cladosporium*, *S. aureus* and *Bacillus subtilis* [77].

Garcinielliptone F (101) and I (102) were isolated as colorless oils from the fresh seeds of *Garcinia subelliptica*. Silica gel column chromatography of chloroform extract using hexane-ethyl acetate gradient yielded garcinielliptone F; with benzene-ethyl acetate-methanol yielded garcinielliptone I. Garcinielliptone F showed potent enzyme inhibitory effects and garcinielliptone I exhibited inhibition of nitric oxide production [78]. 2,4-Diacetylphloroglucinol (36) obtained from symbiotic cultures of *Pseudomonas fluorescens* can also be used as anti-bacterial and anti-fungal agents [79].

Another patent describes extraction and isolation procedure of seven novel phloroglucinol compounds possessing angiotensinase inhibitory activity from *Eucalyptus macrocarpa* and *Eucalyptus globulus* leaves [80]. Another patent gives the extraction and isolation of nine phloroglucinol-terpene adducts namely macrocarpal A (103), B (104), C (105), D (106) and five similar compounds (107 – 111) from dried and ground leaves of *E. globulus*. Steam distillation was carried out to remove the essential oils and remaining plant material was extracted with polar organic solvents. Individual compounds were purified by column chromatography and were patented as anti-caries and anti-periodontopathic agents [81,82]. β-D-glucopyranoxy-phloroglucinol (91), another phloroglucinol compound derived from natural sources, showed anti-microbial properties against dental carious bacteria. It can also be used as an anti-microbial agent for food and drinks and as anti-fungal agent for fruits and vegetables during storage and transportation [83].

There is a patent thoroughly describing the isolation of prenylated and acylated phloroglucinols from hops. There are three major *a*-acids present in whole plant of *H. lupulus*, namely, humulone (53), cohumulone (54) and adhumulone (55) along with iso-*a*-acids, namely, isohumulone (70)

isochumulone (71) and isoadhumulone (72). For isolation of α -acids, acetone extract was prepared by percolation. A portion of this syrup was dissolved in 0.5% acetic acid in methanol and developed on an ion exchange resin Dowex-1. Gradient elution was done using methanol-acetic acid and fractions were monitored by TLC on silica gel using phosphomolybdic acid as visualizing reagent. The fractions giving yellow green color were pooled and further purified using gel filtration on sephadex LH20 to yield 170 mg of α -acids per 100 gm of hops. Bone resorption inhibiting activity was determined by Pit formation assay using the bone cells of ICR mice. α -acids inhibited bone resorption in a dose-dependent manner, ID_{50} being 1.2×10^{-9} M [35].

The isolation procedure of phloroglucinol compounds present in *M. japonicus* is also patented. Two compounds namely mallo-tojaponin (112) and dibutyrylmallotojaponin (113) were isolated from the plant and can be used for their anti-allergic and anti-inflammatory properties [84]. A similar patent describes the extraction and isolation of two phloroglucinol derivatives from the fruit skin of *Mallotus philippensis* and their use as anti-allergic agents [85] (see Figure 4 for structures 97–113).

There are several patented processes for preparation of hyperforin from *Hypericum perforatum*. Hyperforin was obtained by high pressure extraction of flowering tops of *Hypericum perforatum* using carbon dioxide. The extract so formed contained 35% of hyperforin. This extract was dissolved in n-heptane previously saturated with methanol and again extracted into methanol. The methanol phase was dried under vacuum and hyperforin isolated by preparative HPLC. Cognitive effects of hyperforin were demonstrated by conditioned avoidance in rats. Hyperforin and *Hypericum* extract were administered to rats orally for a period of 7 days and it was observed that learned behavior is forgotten less frequently by treated rats after a 10 day pause in the treatment [86]. The mechanism by which hyperforin alleviates the symptoms of depression is also described. It was discovered that hyperforin inhibited serotonin uptake in mouse brain synaptosomes and human platelets by elevating intracellular sodium. Hyperforin was the first anti-depressant known to act by influencing sodium ion concentration [87].

In another process of isolation of hyperforin, dried flowers of *H. perforatum* were macerated with methylene chloride-acetone (50:50) for 24 h at 20°C. The syrup obtained after three such extractions was dried under vacuum and solubilized in ethanol–water (60:40) at 50°C. It was stirred vigorously and filtered through 5 μ filter. The paste obtained after cooling contained about 14% of hyperforin [88]. In another simple process, dried flowering tops of *Hypericum* were extracted using a compressed gas above its critical point. The percentage of hyperforin varied between 20 and 50% depending on temperature, pressure and the extracting gas used. The extract obtained also contained terpenes, other phloroglucinols, carotenoids, phytosterols, fatty acids, cuticular waxes and xanthenes. These components are thought to increase the shelf life of extract itself and the formulations

prepared from it [45]. Hyperforin and related compounds are relatively unstable during long storage conditions; there is a patented process to increase the stability of *Hypericum* extracts and, therefore, the yield of active compounds. Ground plant material was extracted with 70% v/v ethanol at 55°C in an inert atmosphere and 0.1% ascorbic acid is added to the extract after removal of exhausted drug by centrifugation. The solution was intensively stirred for 10 min under nitrogen and dried under vacuum. Hyperforin content of the resultant residue was 0.9%. The yield of hyperforin further increased to 1.7% when the extraction was carried out in dark and using ascorbic acid palmitate as antioxidant. Hyperforin present in extracts, concentrates and medicaments can be stabilized by means of complexing agents [89,90].

Another patent describes an improved method of enriching and purifying hyperforin and adhyperforin from crude extracts without really affecting the stability of these compounds. These compounds were converted to corresponding salts of formula $\{A\}_m\{B\}^{p+}$ where A is anion of hyperforin or adhyperforin and m is whole number from 1 to 3 and B is ion of an alkali metal or ammonium ion of salt forming nitrogen base of general formula $\{N(R_1)(R_2)(R_3)(R_4)\}^{p+}$. Here, $R_1 - R_4$ can be any alkyl or aryl group. In the method given, carbon dioxide extract containing 20 – 80% hyperforin and adhyperforin was dissolved in a polar solvent such as heptane or hexane under inert atmosphere and this solution was added to the solution of component B taken in the same solvent. Crystalline salts of hyperforin and adhyperforin precipitate on removal of solvent under vacuum. Crystalline ammonium salts of hyperforin and adhyperforin were obtained as white powders [72].

Hyperforin can also be extracted by polar solvent extraction or biphasic extraction from the plant material. Further purification by acid base treatment gave an enriched extract that contained about 65% of hyperforin. Chromatographic purification using open column of silica gel and then HPLC gave 95% pure hyperforin [91,92]. A similar Chinese patent gives a simple, practical and low-cost procedure for the extraction of hyperforin and its use as an HIV inhibitor [93].

Hyperforin can also be isolated from an enriched extract as a complex with mineral salts. The stability of hyperforin can be increased by drying the crude extract at low temperature in a suitable pH range [94]. Polyvinylpyrrolidone is also used to remove hypericin selectively from the extracts to render them rich in hyperforin [95].

4. Conclusions and future perspective

We have reviewed existing patents on phloroglucinol compounds covering their use in various pharmaceutical compositions and their isolation processes from natural sources. We believe that phloroglucinols have been an under-explored class of natural products, and after surveying the patents, it seems that this class holds great potential for development of molecules

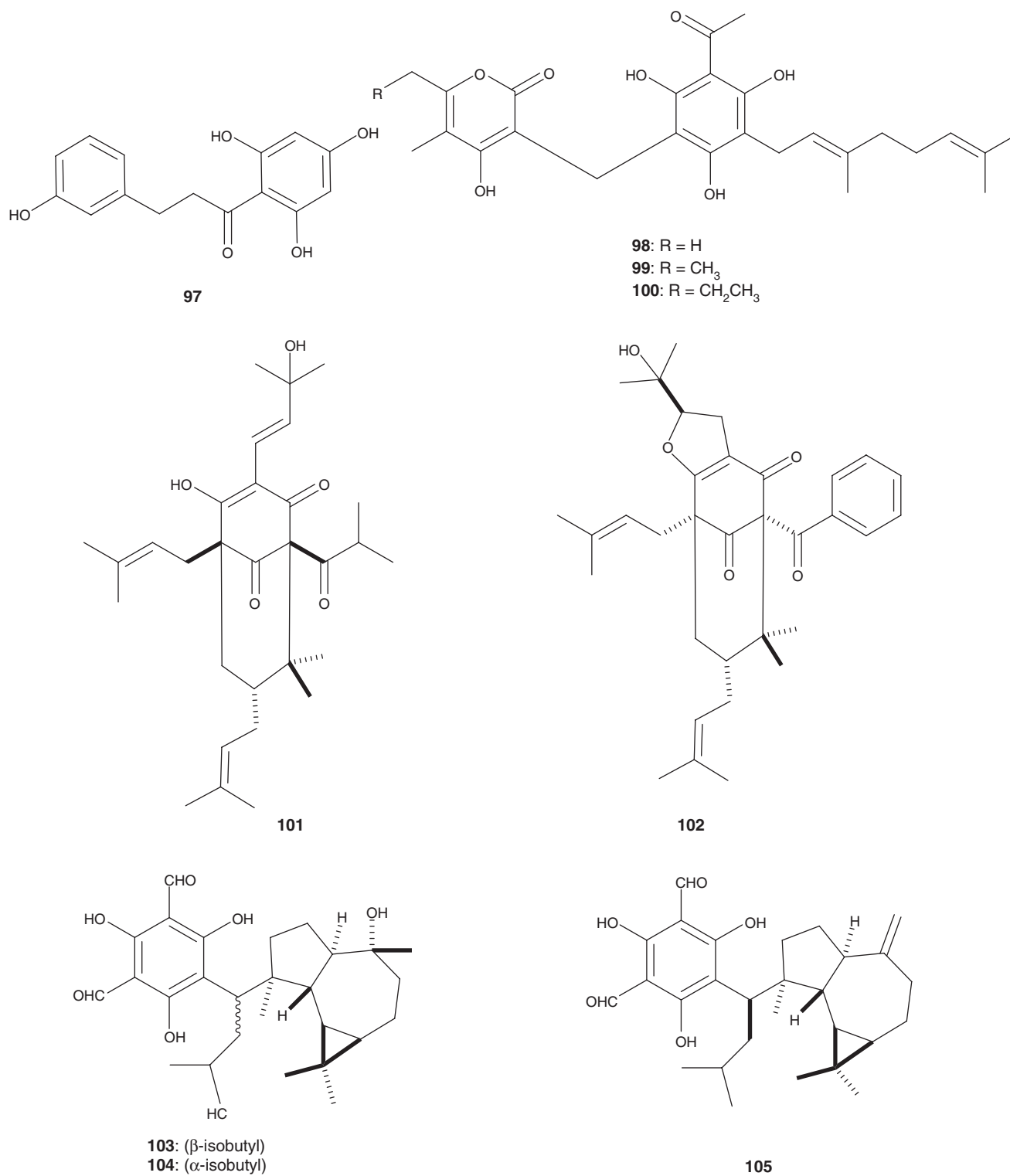


Figure 4. Naturally occurring phloroglucinol compounds.

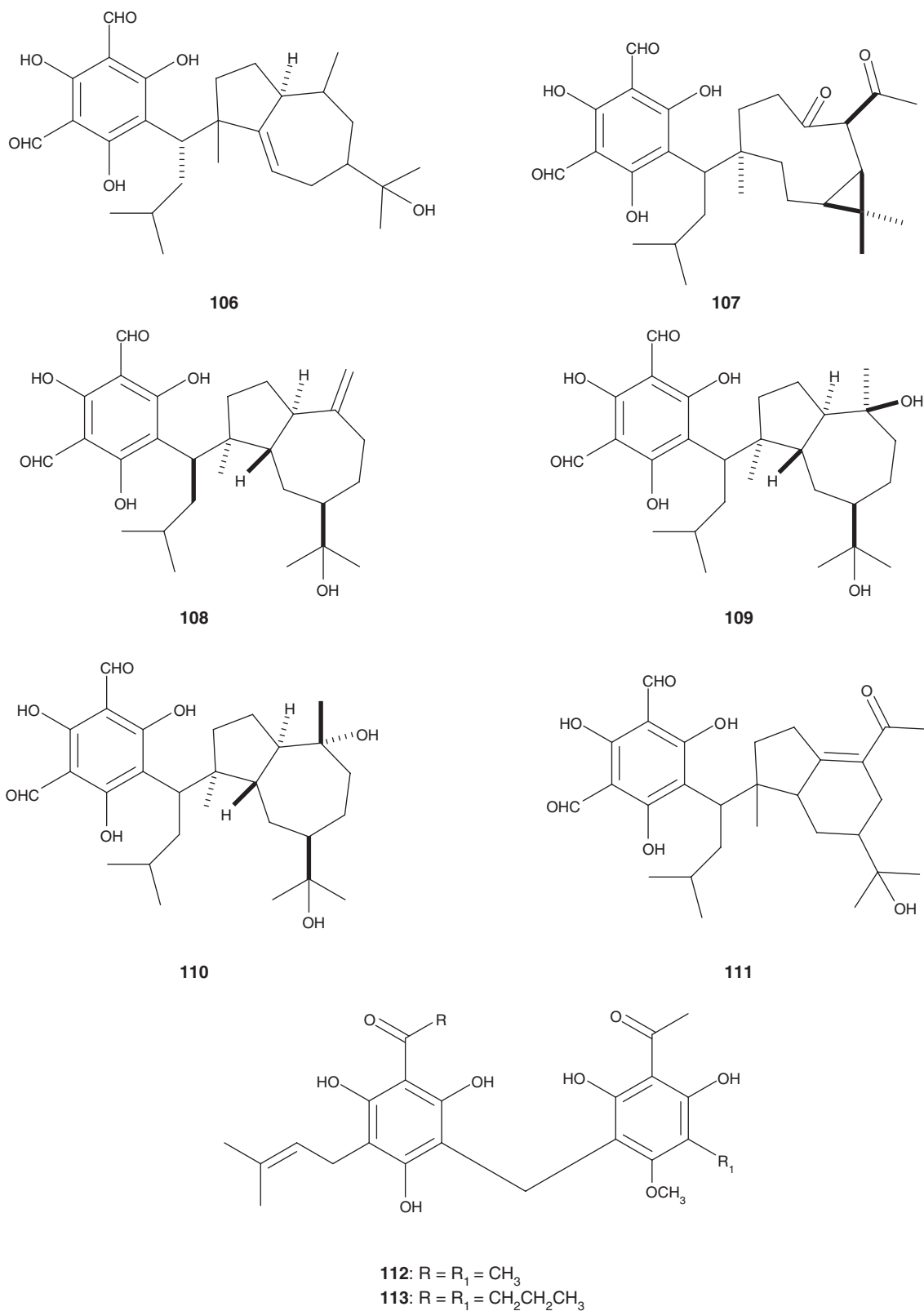


Figure 4. Naturally occurring phloroglucinol compounds (continued).

in various therapeutic areas. A search on the internet showed that few of the compounds/herbs discussed in this review are commercially available and used for medicinal purposes. *Hypericum* herb with a market value of > \$1 billion (the figures vary) is the most popular herbal product containing phloroglucinol compounds. Humulex containing humulone, isohumulone and cohumulone is available commercially in form of tablets for mild pain relief [96]. Psoralens are an important constituent of psoriasis therapeutics [97]. Phloroglucinol is available by trade name Spasfon in form of tablets. Spasfon, a pain drug approved in France for biliary/urinary tract spasm and irritable bowel syndrome accounted for ~ 150 million Euros in 9 months alone in 2001 by Cephalon, Inc. (Frazer, PA, USA). It is also used in painful periods and contraction of uterus during pregnancy and is a very commonly used oxidant and colorant in hair dyes. The aminoketone derivative of phloroglucinol, buflomedil hydrochloride, is also marketed in form of tablets and injections.

5. Expert opinion

Although the use of phloroglucinol compounds in medicine was known since the middle of past century, their scope has been limited mostly to laboratory experimentation only. Phloroglucinol has proven to be effective as an anti-spasmodic agent with patents appearing as recent as in 2002. Its safety and efficacy profiles have also been thoroughly investigated; however, there are a very few phloroglucinol-containing preparations in market that find use in clinics. Similarly, ether derivatives of phloroglucinol were found to show anti-spasmodic and hypercholeretic activity at very low doses. Many patents were granted in the 60s of the 20th century for synthesis of such compounds. Formulations containing these compounds have been tested *in vitro* and/or *in vivo* for a wide range of biological activities. These off patented technologies give synthetic insights to many active and therapeutically useful molecules and provide potential for future exploitation of these molecules. As is clear from the structures, most molecules contain moieties that can be easily transformed or coupled to other therapeutically useful molecules.

Many natural phloroglucinols with potential therapeutic applications have been isolated from plant and microbial sources. Compounds such as aspidinol and methylene bis-aspidinol have shown anti-malarial activity in nano molar range. Another natural compound caespitate has significant anti-microbial and anti-tubercular activity. These compounds were formulated to various dosage forms and evaluated for biological activity but despite their potential for development as drug molecules or leads they have largely been ignored. As generally observed in natural products research, the compounds are isolated in very small amounts, their structures explained and biological assay performed in the field of interest and the paper is published. For most new natural

products reported in literature, patents are not taken. Further, the biogenetic pathways of most of these naturally occurring phloroglucinol molecules can be visualized, thus giving a handle to introduce therapeutic scaffolds into the basic phloroglucinol moiety and also for planning biomimetic synthesis of analogues with further active moieties introduced into those. There may exist a goldmine of such molecules if libraries are generated and evaluation done in therapeutic areas other than originally reported for those compounds.

Some of these natural molecules can be lead candidates or natural product templates for synthesis or semi-synthesis of other more efficacious molecules. This is an area that we believe can be exploited to generate more active compounds for future commercialization and may hold great promise in the coming years. This is exemplified by our recent work in which *S*-euglobals were shown to possess anti-leishmanial activity. Euglobals are phloroglucinol-monoterpene adducts isolated from various species of *Eucalyptus* and exhibit Epstein–Barr virus early antigen inhibitory activity. Based on the proposed biogenesis of these compounds involving inter-molecular Diels–Alder reaction between *O*-quinone methide generated from formylated phloroglucinols and monoterpenes present in eucalyptus, we synthesized related compounds using monoterpenes not reported in naturally occurring euglobals. These synthetic euglobals named *S*-euglobals demonstrated anti-leishmanial activity *in vitro* [98–100]. Encouraged by this new activity of *S*-euglobals, the euglobal rich fraction from eucalyptus was evaluated *in vivo* in golden hamsters for anti-leishmanial activity. The positive results obtained suggest euglobals to be a new and promising class of anti-leishmanial compounds (Singh, unpublished results).

Another natural phloroglucinol, 5-methoxypsoralen, has been used in treatment of psoriasis. Patents encompassing its formulations, synthesis and utilization have been granted. Alternative healthcare systems use psoralens for skin disorders and some preparations containing these compounds are available in markets.

Hyperforin is a thoroughly exploited natural phloroglucinol molecule. Many synthetic and semi-synthetic derivatives of hyperforin have been prepared and tested for various biological activities such as anti-depressant, anticancer and antioxidant. Use of hyperforin in Alzheimer's disease is well documented. Many process patents for isolation and enrichment of hyperforin from crude extract of *Hypericum* spp. do exist.

Many of these compounds have potential for further development as drug molecules. Isolation, synthetic strategies and formulations for testing of biological activities have already been patented. These technologies have to be investigated further so that leads could be generated and optimized for use in the therapeutic areas. To cite a few examples, molecules such as aspidinols, psoralen, mallotojaponin, and caespitate hold promise for further studies.

Acknowledgement

The authors are thankful to the Director, NIPER and HOD, Department of Natural Products, NIPER for support.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as of interest (●) to readers.

- Singh IP, Bharate SB. Phloroglucinol compounds of natural origin. *Nat Prod Rep* 2006;23:558-591
- Lafon L. Pharmaceutical compositions containing phloroglucinol. GB904955; 1962
 - **Demonstrates the anti-spasmodic activity of phloroglucinol commercially available as spasfon.**
- Bennis A, Serrano J-J, Bennis F. Pharmaceutical compositions for oral administration of phloroglucinol and preparation thereof. HK1045937; 2004
 - **Demonstrates the anti-spasmodic activity of phloroglucinol commercially available as spasfon.**
- Bennis A, Serrano J-J, Bennis F. Pharmaceutical compositions for oral administration of phloroglucinol and preparation thereof. US6716884; 2004a
 - **Demonstrates the anti-spasmodic activity of phloroglucinol commercially available as spasfon.**
- Orsymonde. Pharmaceutical compositions comprising phloroglucinol ethers. GB1272562; 1972
- Ham IG, Kim GT, Kim SG, et al. New spasmolytic agent containing phloroglucinol as effective ingredient. KR20020045948; 2002
- Dubois J, Frayssinet L. Compositions containing ibuprofen and phloroglucinol. FR2735025; 1966
- Lafon V. Improvements in or relating to phloroglucinol and derivatives. GB1276097; 1972
- Vaille M. Improvements in or relating to phloroglucinol and derivatives. GB1271519; 1972
- Lafon L. Improvements in or relating to phloroglucinol and derivatives. GB1262785; 1972
- Chan JA, Westley JW. Anti-viral compositions. WO9109595; 1991
- Tada M, Seta A, Kuribayashi Y, et al. Anti-Herpes virus agent. JP4128220; 1992
- Tada M, Seta A, Kuribayashi Y, et al. Anti-Herpes virus agent. JP4124129; 1992a
- Kapadia GJ. Antimalarial agents. US5807898; 1998
- Meyer JJM, Mathegka ADM. Phloroglucinol compounds. WO0123342; 2001
 - **Demonstrates anti-tubercular activity of caespitate.**
- Courtney WJ. Anti-microbial composition comprising *Leptospermum scoparium* and *Melaleuca alternifolia* oils. WO0027206; 2000
- Nagayama K, Hirayama I, Nakamura T, et al. Anti-bacterial agents based on phlorotannins. JP2001199881; 2003
- Choi JS, Jung HY, Jung JH, et al. Composition comprising phlorotannin isolated from extract of *Ecklonia stolonifera* Okamura having anti-oxidant activity, wherein the phlorotannin effectively scavenges free radicals. KR20040095125; 2004
- Miyazaki T, Shiojiri M. Urease inhibitor. JP2006241039; 2006
- Nagayama K, Kimura T, Hirayama I, et al. Antiviral agent. JP2004189648; 2004
- Byun YR, Hwang JK, Park JH. Antibacterial agent and oral composition containing panduratin derivatives for prevention and treatment of dental carries and periodontitis. KR20030060440; 2003
- Tobe H, Muraki Y, Arai S. Therapeutic agent for infectious disease with methicillin-resistant *Staphylococcus aureus*. JP7228527; 1995
- Tagashira M. Utilization of humulones having antioxidant action. JP6312924; 1994
- Tagashira M. Utilization of mallophenone having antioxidant action and its production. JP8026981; 1996
- Broadbent JL, Bowden K, Ross WJ. 2,4-Diloweralkanoyl phloroglucinol derivatives. US3467715; 1969
- Goupil J-J. 5-Methoxy-psoralen used to treat psoriasis. US5360816; 1994
 - **Demonstrates photodynamic activity of psoralens.**
- Goupil J-J. 5-Methoxy-psoralen as new medicament and process for the synthesis thereof. US5405868; 1995
 - **Demonstrates photodynamic activity of psoralens.**
- Puybaret C, David B, Charveron M, et al. Extract of myrtle containing acylphloroglucinols, especially myrtucommulone B', for treating psoriasis and keratinization disorders. FR2783425; 2000
- Bertini CS. Drug and method for increasing the microcirculatory flow-rate and volume in the capillaries to the skin. US5280020; 1994
- Breton L, Girerd F, Renault B. Promoting epidermal renewal with phloroglucinol. US006054137A; 2000
- Lee JN, Jeong JH, Kee B, et al. Cosmetic composition comprising extract from *Mallotus japonicus* for improving skin wrinkle. US2007053863A1; 2007
- Leon JLMD. Cell regenerator. MX03000020; 2005
- Lescroart P. Nerve damage. WO2005107744; 2005
 - **Buflo-medil - commercially available drug.**
- Lescroart P. Aminoketone derivatives of phloroglucinol for the treatment of motor neuron disease. GB2414665; 2005
- Tobe H, Kitamura K. Pharmaceutical composition for treating osteoporosis. EP0677289A2; 1995
 - **Humulones - commercially available as Humulex for mild pain relief.**
- Lafon L. New phloroglucinol derivatives, their method of preparation and their use as pharmaceuticals. GB2004883; 1979
- AbbottLab. Tri-cyclic compounds derived from phloroglucinol. GB1510431; 1978
- Maillard J, Vincent M, Delaunay P. Therapeutic compositions containing phloroglucinol derivatives, new derivatives

- of phloroglucinol and process for preparing the same. GB1067575; 1967
39. Boucard M. Improvements in or relating to 2,4,6-trihydroxy-benzoic acid and its derivatives. GB042835; 1966
 40. Yamamoto K, Saegusa H, Hiramitsu M, et al. Apoptosis inducer. JP2001199881; 2001
 41. Tada K, Chiba K, Kojima H, et al. Allergic disorder therapeutic agent. JP4108730; 1992
 42. Tada K, Chiba K, Kojima H, et al. 2,6-diacyl-4,4-dialkylcyclohexane-1,3,5-trione and allergic disorder therapeutic agent with the same as active ingredient. JP108729; 1992
 43. Bae JH, Lee CH, Lee HU, et al. Composition to improve sexual dysfunction. KR2004008166; 2004
 44. Strong AH, Levy J, Huber G, et al. Formulation for visual acuity amelioration by photodynamic therapy of eye. JP2006273853; 2006
 45. Newmark T, Schulick P. Herbal composition and method for promoting emotional well-being. US2002107172; 2002
 - **Isolation procedure for hyperforin.**
 46. Castor TP. Formulations for hyperforin-enriched hypericum fractions. US2006240098; 2006
 47. Duan ZG. An oral administered medicine for treating depression – Jin Yu Kang. CN875954; 2006
 48. Zhang M, Duan Z, Guo S. Jinyukang – oral medicine for curing depression. CN408396; 2003
 49. Bombardelli E. Compounds useful in the therapy of Alzheimer's disease and formulations containing them. US2007010507; 2007
 50. Timothy HMD. Combinations of St. John's Wort and fatty acids. WO0033831; 2000
 51. Bombardelli E, Morazzoni P, Riva A, et al. Hyperforin derivatives, the use thereof and formulations containing them. HK1075654; 2007
 52. Bombardelli E, Morazzoni P, Riva A, et al. Hyperforin halogenated derivatives, the use thereof and formulations containing them. HK1075653; 2006
 53. Bombardelli E, Morazzoni P. Hyperforin derivatives, the use thereof and formulations containing them. HK1036791; 2006
 54. Erdelmeier C, Grethlein E, Lang F, et al. Stable dry extract of *Hypericum perforatum* L., Saint John's Wort – with stabiliser and higher hyperforin content, used to treat depression and psycho-vegetative illness, obtained by solvent extraction etc. DE19619512; 1997
 55. Erdelmeier C, Grethlein E, Lang F, et al. Stable extract of *Hypericum perforatum* L., a method for producing the same, and corresponding pharmaceutical preparations. US6241988; 2001
 56. Erdelmeier C, Grethlein E, Lang F, et al. Stable extract of *Hypericum perforatum* L., process for preparing the same and pharmaceutical compositions. US6280736; 2001
 57. Erdelmeier C, Grethlein E, Lang F, et al. Stable extract of *Hypericum perforatum* L., processes for its preparation and pharmaceutical compositions. TW471969B; 2002
 58. Chatterjee SS, Erdelmeier C, Stumpf H. St. John's Wort dry extract, process for its preparation and its use. EP0599307; 1994
 59. Bombardelli E, Morazzoni P. Hyperforin derivatives, the use thereof and formulations containing them. SI1086071T; 2006a
 60. Luo M. *Hypericum perforatum* L. phenolic acid and injection thereof preparation method CN1883520; 2006
 61. Simon JC, Schempp CM, Schoepf E, et al. Hyperforin as cytostatic agent and hyperforin ointment or cream as application form. US7179495; 2007
 62. Schempp CM, Simon J, Schoepf E. Use of hyperforin for treating cancer, or in topical compositions optionally together with hypericin for treating e.g. inflammatory skin disease, skin aging or bacterial skin infections. DE19854446; 2000a
 63. Schempp C, Simon JC, Simon-haarhaus B, et al. Hyperforin as cytostatic agent and hyperforin ointment or cream as application form. EP1275382; 2003
 64. Schempp C, Simon-Haarhaus B, Simon JC. Use of hyperforin for treating cancer, or in topical compositions optionally together with hypericin for treating e.g. inflammatory skin disease, skin aging or bacterial skin infections. DE19913333; 2000
 65. Irion KM, Beck G. Use of St. John's Wort constituents for photodynamic diagnosis and/or therapy of mouth and malignant brain disorders. DE19950933; 2001
 66. Roots I, Krusekopf S. Use of hyperforin or St. John's Wort in the treatment and prevention of metabolic syndrome. EP1774974; 2007
 67. Schempp C, Jocher A, Engel K, et al. Pharmaceutical compositions from beard lichen (*Usnea barbata*) and St. John's Wort (*Hypericum perforatum*) and their use. WO2005099728; 2005
 68. Joachim H. Improved and stable extract from *Hypericum perforatum* L. MX03005657; 2004
 69. Schempp CM, Simon JC. Use of hyperforin and derivatives thereof for inhibiting angiogenesis. WO03094945; 2003
 70. Werz O, Albert D, Steinhilber D, et al. Use of hyperforin or St John's Wort extracts against anaphylactic states of shock and for maintaining and improving bone health. WO03053456; 2003
 71. Sutherland SK, Shan JJ. Antioxidant and Fe²⁺-chelating properties of herbal extracts. WO2007045959; 2007
 72. Chatterjee SS, Erdelmeier C, Klessing K, et al. Stable hyperforin salts, method for producing same and their use in the treatment of Alzheimer's disease. US6444662; 2002
 73. Chancellor MB, Tyagi P, Kaufman J. Active agent and formulations to minimize or alleviate bladder urgency and irritation and/or to enhance sexual function. US2007028930; 2007
 74. Rizzi GP. Novel dihydrochalcone sweetener compositions. US3932678; 1976
 75. Boehringer. Process for the production of preparation suitable as a blood plasma substitute. GB736354; 1955
 76. Roussel U. Improvements in or relating to the purification of vitamin B12. GB833067; 1960
 77. Tomas-lorente F, Iniesta-Sanmartin E, Tomas-Barberan F, et al. Phloroglucinol derivatives. DE3907543; 1990
 78. Lin C-N, Wang J-P, Weng J-R. Anti-inflammatory and cure for ageing, Alzheimer's disease on phloroglucinol derivatives. US2006205808; 2006
 79. Aino K, Maki H, Shimizu K, et al. Method for producing antimicrobial

- substance: 2,4-diacetylphloroglucinol. JP7187938; 1995
80. Saeki T, Osawa K, Yasuda H. Angiotensinase inhibitor and angiotensinase inhibitory food/drink with the same as active ingredient. JP11060498; 1999
 81. Osawa K, Yasuda H. Anti-cariious and anti-periodontopathic agent and composition for oral cavity containing the same. JP8109118; 1996
 82. Osawa K, Yasuda H. Preventing and therapeutic agent for throat inflammation and hemolysin and composition for oral cavity containing the same. JP8259452; 1996
 83. Tamaoki Y, Kato T, Miyake Y. Anti-microbial agent. JP8027005; 1996
 84. Kitanaka S, Daikonya A, Arisawa M, et al. Anti-allergic agent and anti-inflammatory agent using fluoroglycinol, derivative. JP2003073265; 2003
 85. Kitanaka S, Daikonya A, Katsuki S. New phloroglucinol derivative, composition given by using the same, and anti-allergenic agent. JP2003055369; 2003
 86. Chatterjee SS, Erdelmeier C, Noeldner M. Use of hyperforin and hyperforin-containing extracts in the treatment of dementia diseases. US6322824; 2001
 87. Singer A, Wonnemann M. Treatment of depression by using compounds which elevate the intracellular sodium concentration EP1034782; 2000
 88. Quintanilla AE, Ramirez BA, Abernd A, et al. Hypericum Perforatum L. oleoresin, procedure for obtaining it and uses of it. US2002197337; 2002
 89. Stumpf K-H, Erdelmeier C, Oschmann R, et al. Stable preparations with hyperforin. EP1126882; 2001
 90. Stumpf K-H, Erdelmeier C, Oschmann R, et al. Stabile Zubereitungen mit Hyperforin. DE19903570; 2000
 91. Yang D, Wang F, Huang S. Extracting and purifying method for Hypericum perforatum component in plant. CN1392130; 2003
 92. Yang D, Wang F, Huang Y. Process for preparing Hypericum perforatum extract. CN1392129; 2003
 93. Li X. Extracting technology for medicinal hyperforin as plant product. CN1473812; 2004
 94. Lanquetin M, Rougaignon C, Viant P. Extraction of active principles from plants to increase the rate and to ensure the stabilization of acylphloroglucinol, comprises subjecting the extracts in a suitable pH zone followed by drying in vacuum at low temperature on zeolite. FR2894829; 2007
 95. Erdelmeier C, Grethlein E, Lang F, et al. Stable extract of Hypericum perforatum L., processes for its preparation and pharmaceutical compositions US2002031560; 2002
 96. Available from: www.herbalremedies.com
 97. Available from: <http://www.researchandmarkets.com>
 98. Bharate SB, Bhutani KK, Khan SI, et al. Biomimetic synthesis, antimicrobial, antileishmanial and antimalarial activities of euglobals and their analogues. Bioorg Med Chem 2006;14:1750-60
 99. Bharate SB, Khan SI, Yunus NAM, et al. Antiprotozoal and antimicrobial activities of O-alkylated and formylated acylphloroglucinols. Bioorg Med Chem 2007;15:87-96
 100. Bharate SB, Khan SI, Tekwani BL, et al. S-Euglobals: Biomimetic synthesis, antileishmanial, antimalarial, and antimicrobial activities. Bioorg Med Chem 2008;16:1328-36

Affiliation

Inder Pal Singh^{†1} PhD,
 Jasmeen Sidana² MS Pharm,
 Parikshit Bansal³ PhD &
 William J Foley⁴ PhD
[†]Author for correspondence
¹Associate Professor
 National Institute of Pharmaceutical Education
 and Research (NIPER),
 Department of Natural Products,
 Sector-67, S.A.S. Nagar, Punjab 160062, India
 Tel: +91 172 2214683 extn. 2144;
 Fax: +91 172 2214692;
 E-mail: ipsingh@niper.ac.in; ipsingh67@yahoo.com
²PhD student
 National Institute of Pharmaceutical Education
 and Research (NIPER),
 Department of Natural Products,
 Sector-67, S.A.S. Nagar, Punjab 160062, India
³Assistant Professor
 National Institute of Pharmaceutical Education
 and Research (NIPER),
 Intellectual Property Rights (IPR) Cell,
 Sector-67, S.A.S. Nagar, Punjab 160062, India
⁴Professor and Head
 The Australian National University,
 School of Botany and Zoology,
 Canberra 0200, Australia