CHAPTER 1

Introduction

1.1. Medicinal natural products in history

The knowledge of natural product with medicinal property is as ancient as human civilization and nearly every civilization has accumulated experience of their use. The earliest record of natural products has come from ancient Mesopotamia, circa 2600 BC, and is written on clay tablets in cuneiform. Almost 1000 plants and plant derived substances, such as oils from *Cupressus sempervirens* (Cypress) and *Commiphora* species (myrrh) were documented which are still used today for treating coughs, colds and inflammation. The well known document of Egyptian pharmaceutical record is the Ebers Papyrus (1500 BC) which contains 700 plants based drugs and including formulas such as gargles, pills, and ointments. The Chinese are leaders for using natural products. The most primitive Chinese medical book, Wu Shi Er Bing Fang (1100 BC) contains 52 prescriptions followed by works such as the *Shennong* Herbal (~100 BC; 365 drugs), and the *Tang* Herbal (659 AD; 850 drugs). Documentation of traditional Indian medicine i.e. Ayurveda starts from about 1000 BC (Susruta and Charaka). The Greek scientist and philosopher, Theophrastus (~300 BC) dealt with the medicinal qualities of herbs while the collection, storage, and use of medicinal herbs were recorded by Greek physician Dioscorides (100 AD). Galen (130-200 AD) published at least 30 books on pharmacy and medicine in Rome. From the 5th to 12th centuries, the monasteries in England, Ireland, France, and Germany preserved this knowledge but Arabs were expanding their knowledge by including their own resources with Greco-Roman expertise together with Chinese and Indian herbs unknown to the Greco-Roman world [1].

1.2. Natural products and drug discovery

Various natural products contain different compounds and modern chemistry has provided the tools to purify various compounds and to determine their structures. In 1805, the German pharmacist Friedrich Wilhelm Sertürner isolated morphine from opium. This is the first naturally derived medicine commercialized by Merck in 1826. In fact, Western pharmaceutical companies quickly began to use purified natural products as ingredients for making drugs, rather than crude extracts. In addition, molecular structure determination of many compounds encouraged the chemistry to synthesize them rather than isolating them from natural sources which lowered drug production cost. Hence, a large number of natural compounds were identified, analyzed and synthesized: strychnine and brucine from *Strychnos nux-vomica* (strychnos), nicotine from *Nicotiana tabacum*, atropine from *Atropa belladonna* etc. In the 20th century, the discovery of penicillin from the mould *Penicillium notatum* gave physicians a powerful weapon in their battle against antibacterial diseases. Chemists have now modified the structure of natural compounds to suppress or enhance certain characteristics such as solubility, efficiency or stability in the human body [2]. Newman pointed out that approximately 60% of the drugs that are now available-including household names such as artemisinin, camptothecin, maytansine, paclitaxel, penicillin, reserpine and silibinin were either directly or indirectly derived from natural products [3].

1.3. Co-evolution

The important question is that why so many natural products are useful to human health and one explanation is that it is the result of long-term co-evolution within biological communities: simultaneous evolution of multiple interactive species. Hence, those natural compounds are able

to suppress the growth of bacteria; such molecules can also exert same effects in humans and are now used as antimicrobial drugs in medicine [4].

1.4. Plant defense mechanism: production of secondary metabolites

The production of secondary plant metabolites may be the most important strategy for plant defense against animal and microbial predators. Though the function of many plant secondary metabolites is not known, we can assume these chemicals may have general or specific activity against key target sites in bacteria, fungi, viruses, or neoplastic diseases [5].

1.5. Terpenoids: secondary metabolites

Terpenoids are the largest and most widespread class of secondary metabolites which occur mainly in plants and lower invertebrates. Traditionally, humans used plant-based terpenoids in the food, pharmaceutical, and chemical industries. A large number of terpenoids show a variety of biological activities such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities [6, 7].

1.5.1. Structural features and classification of terpenoids

The term terpene is generally used to represent only the hydrocarbons $(C_5H_8)_n$ while the term terpenoids represent the hydrocarbons as well as their oxygenated derivatives. The important structural feature of nearly all the terpenoids is their biosynthesis from one monomeric structural unit, isoprene (C_5H_8). Ingold suggested that the isoprene units in terpenoids were joined in head to tail linkages, may be referred to as the special isoprene rule (Figure 1.1). But this rule is not unique as many exceptions occur, e.g., the two halves of carotenoids are joined tail to tail fashion; cryptone, a natural terpenoid, have nine carbon atoms instead of ten and hence isoprene

rule cannot be applied [8, 9]. The C_5H_8 units polymerize and subsequently fix the number and position of double bond. The basic C_5 unit can be combined in many ways; it is not surprising to observe the amount and diversity of the structures [10].

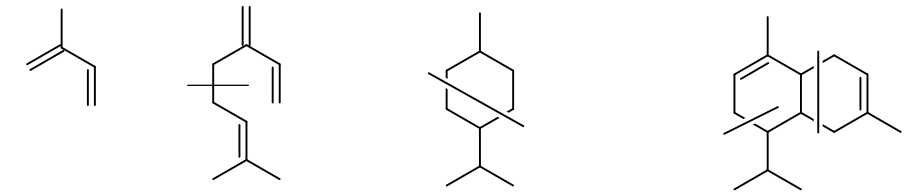


Figure 1.1. Illustration of isoprene rule of Myrcene (two isoprene units), p-Menthane (two isoprene units) and Cadinene (three isoprene units)

The classes of terpenoids found in nature are listed in Table 1.1.

Table 1.1. Classes of terpenoid with their isoprene units and molecular formula

Class	Number of Isoprene units (C_5H_8)	Molecular formula
Hemiterpenoids	1	C_5H_8
Monoterpenoids	2	$C_{10}H_{16}$
Sesquiterpenoids	3	$C_{15}H_{24}$
Diterpenoids	4	$C_{20}H_{32}$
Sesterterpenoids	5	$C_{25}H_{40}$
Triterpenoids	6	$C_{30}H_{48}$
Tetraterpenoids or Carotenoids	8	$C_{40}H_{64}$
Polyterpenoids	n	$(C_5H_8)_n$

Each class of terpenoid may be further subdivided on the basis of the number of rings present in the molecule i.e. monocyclic, bicyclic, tricyclic etc.

1.5.2. Biosynthesis of terpenoids

Plants have tremendous biosynthetic potentials and the fundamental units used in syntheses in the cell are water, carbon dioxide, formic acid (as active formate), and acetic acid (as active acetate). These active compounds form acyl derivatives of coenzyme A. This coenzyme is a complex thiol derivative and is represented as CoA-SH. The acetylcoenzyme A, usually written as CH_3CO-SCoA, may be formed by the condensation between acetic acid and coenzyme A. The conversion of acetyl-coenzyme A (5) into isopentenyl diphosphate (9, IPP) is believed to proceed via acetoacetyl-coenzyme A (6), 3-hydroxy-3-methylglutaryl-coenzyme A (7), and mevalonate (8) shown in Figure 1.2.

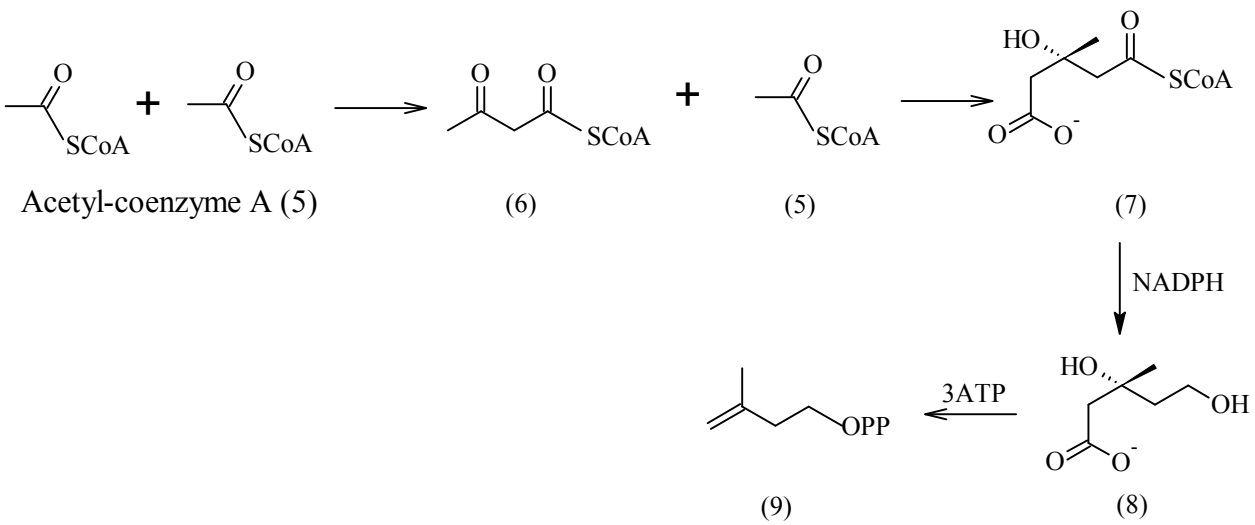


Figure 1.2. Mevalonate pathway for isopentenyl diphosphate (IPP) biosynthesis

This isopentenyl diphosphate is transformed into all different terpenoids found in nature. In the presence of appropriate enzyme, isopentenyl diphosphate (9, IPP) is isomerized to dimethylallyl diphosphate (10). Either of these can be converted into hemiterpenoids (Figure 1.3).

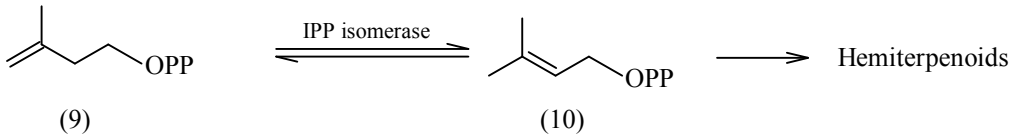


Figure 1.3. Isomerization of isopentenyl diphosphate to dimethylallyl diphosphate

The condensation of dimethylallyl diphosphate (10) with isopentenyl diphosphate (9) under the action of the enzyme prenyltransferase gives geranyl diphosphate (11) which can serve as the precursor for monoterpenoid biosynthesis (Figure 1.4).

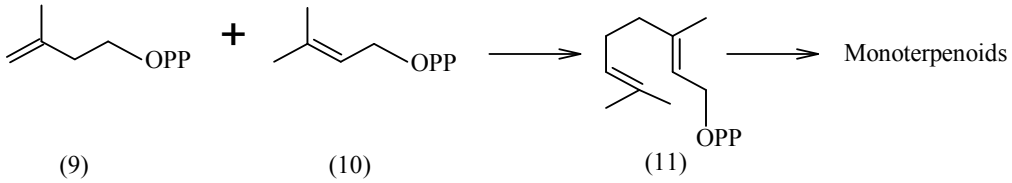


Figure 1.4. General scheme of monoterpenoid biosynthesis

The condensation of geranyl diphosphate (11) with another IPP molecule produces farnesyl diphosphate (12) which can be either channeled into sesquiterpenoid biosynthesis or undergo a tail-to-tail dimerization to form triterpenoids (13) (Figure 1.5).

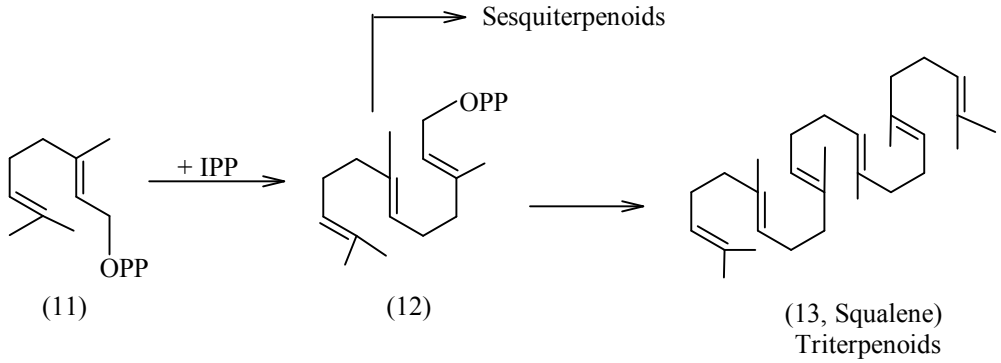


Figure 1.5. General scheme for sesquiterpenoid and triterpenoid biosynthesis

Farnesyl diphosphate (12) can undergo chain elongation with IPP to form geranylgeranyl diphosphate (14) which has three metabolic fates, to form diterpenoids, to undergo chain extension to form geranylfarnesyl diphosphate (15) and tail-to-tail dimerization to tetraterpenoids

(16). Geranylfarnesyl diphosphate (15) is transformed into sesterterpenoids or undergoes chain extension to produce polyterpenoids (Figure 1.6) [6, 11].

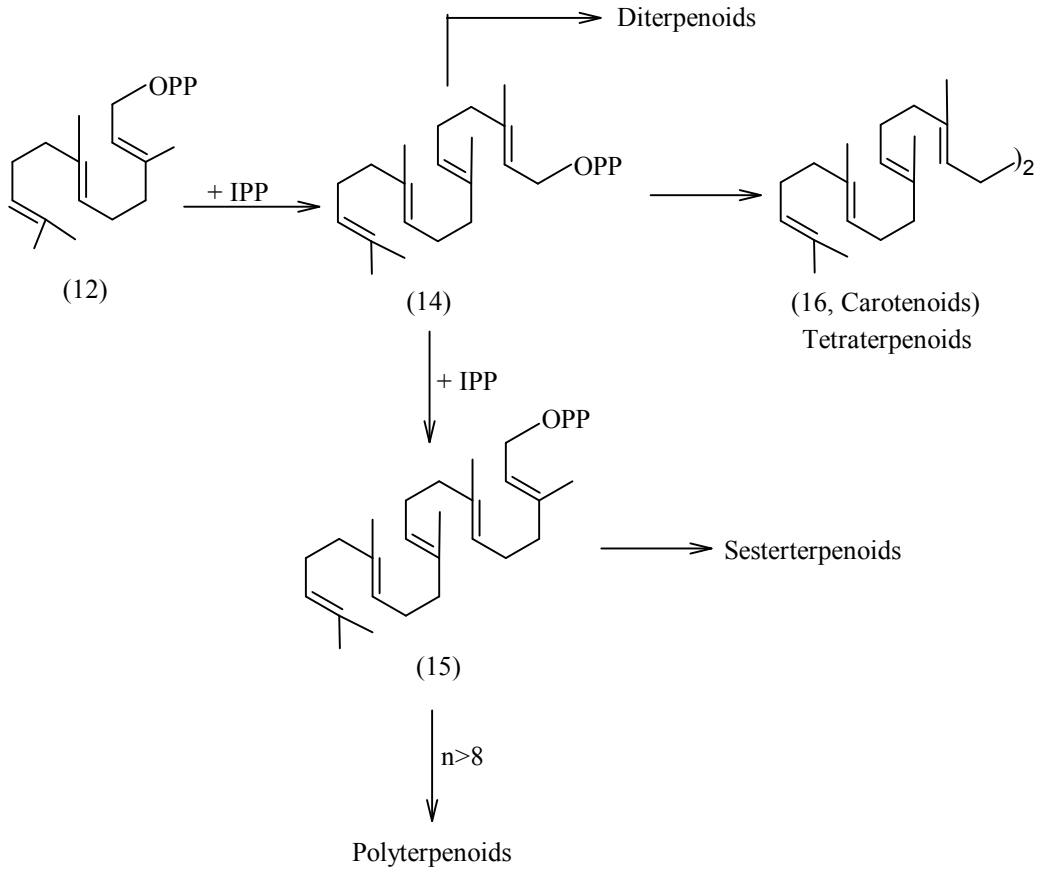


Figure 1.6. General scheme for diterpenoid, sesterterpenoid and polyterpenoid biosynthesis

1.5.3. Extraction and isolation

As the terpenoids are of wide occurrence, they can be isolated by a great variety of methods. The essential oils, which are mixture of lower terpenes (mono and sesquiterpenes), are extracted by subjecting plants to steam distillation. The individual terpenoids from the essential oils are then separated by successive fractional distillation. During fractional distillation, the terpenoid

hydrocarbons distill first followed by their oxygenated derivatives. The sesquiterpenoids are obtained under the distillation of the residue at reduced pressure. More recently, various forms of chromatography, such as column chromatography, preparative HPLC, radial chromatography, have been employed for the isolation and separation of terpenoids [12].

1.5.4. Structural elucidation

The structures of terpenoids are examined by a combination of chemical and spectroscopic methods. However, the recent trend is to determine the structures by the spectroscopic techniques which require small amount of the sample. Nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C and 2D NMR) has been used to elucidate the structure of a new terpenoid including the relative stereochemistry of chiral centres. NMR has also been used to recognize substance comparing with a previously known compound, considering the huge literature data on these metabolites that have been gathered during the past decades. Moreover, the absolute configuration of a terpenoid can be further established by several methods like X-ray analysis, optical rotator dispersion, circular dichroism, exciton chirality, chemical correlation with a compound of known absolute configuration, resolution of racemates, as well as enzymatic reactions [13-15].

1.5.5. Bioactivity of terpenoids

A number of monoterpenoids possess many biological activities. Citral is an acyclic monoterpenoid hydrocarbon which occurs in lemon grass oil present at levels of 65-85%. Citral (3,7-dimethyl-2,6-octadienal) exists in two geometrical isomeric forms: trans citral (citral-a, geranial, 17) and cis citral (citral-b, neral, 18). Citral has good antimicrobial, antibacterial and antifungal activity [16, 17]. Citral also possesses anticancer activity against HeLa, ME-180, NB4

and MCF-7 cell lines in vitro [18-20]. Apoptosis or programmed cell death is suppressed in many diseases including cancer. It may be mentioned that citral induced apoptosis of NB4 cell takes place by decreasing mitochondrial membrane potential. Bcl-2 down-regulation, Bax up-regulation on mRNA level and NF-κB down-regulation on protein level may be involved in the mechanism of the apoptotic effect of citral on NB4 cells [20]. A very recent report on the anti-tumor activity of citral-isomers reveals that geranial is more potent in inhibiting cytotoxicity/tumor-growth in mammalian physiology [21]. The roles of limonene (19), perillyl alcohol (20), carvone (21), and carveol (22) have been investigated in human health due to their chemotherapeutic activities. They inhibit carcinogenesis both in the initiation and promotion/progression stages, and are effective in early and advanced cancers treatment [22-25]. Several monoterpenoids have been studied for their antioxidant activity and γ-terpinene (23) was shown to be an important antioxidant [26]. Monoterpene hydrocarbons such as menthol (24), limonene (19), linalool (25) etc. are used as flavouring agent in foods and beverages [27]. Figure 1.7 shows the structures of the some important monoterpenoids.

Sesquiterpenoids function as the pheromones and juvenile hormons in plants. Farnesol (26), a acyclic sesquiterpene, has anti tumor activity [28]. Because of its anti bacterial activity, it is also used as a deodorant [29]. The bicyclic sesquiterpene β-caryophyllene (27) was shown to be selective agonist of cannabinoid receptor type 2 which is the alternative therapeutic targets for the treatment of anxiety and depression [30]. Abscisic acid (28) is a plant hormone that accelerates the development process in many plants including seed development and dormancy [31]. Some important sesquiterpenoids are shown in Figure 1.8.

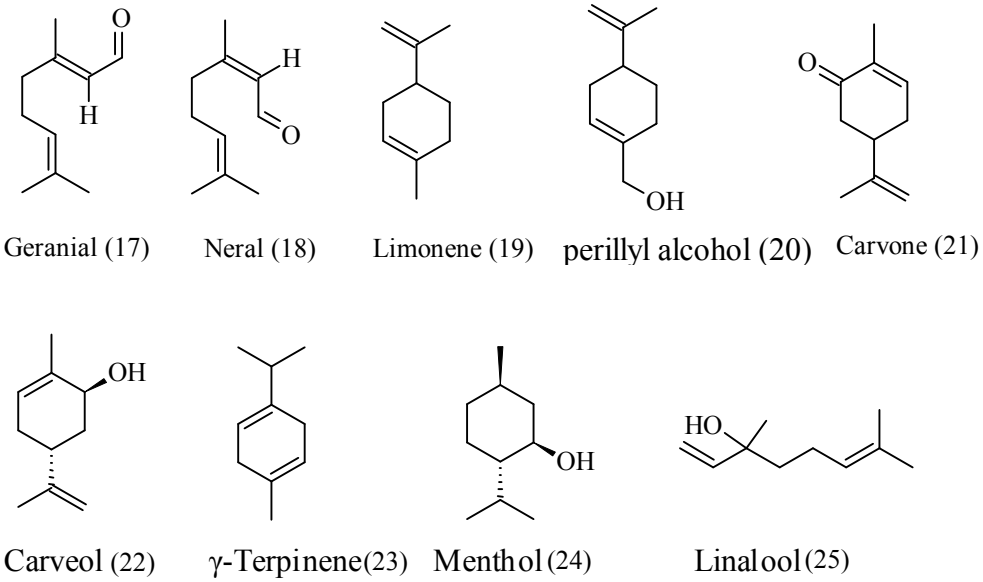


Figure 1.7. Structures of the some important monoterpenoids

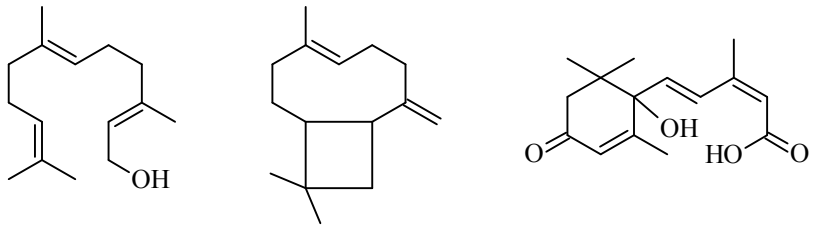
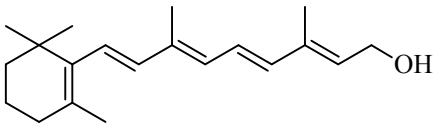


Figure 1.8. Structures of the some important sesquiterpenoids

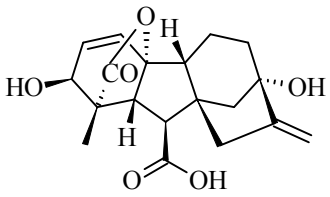
The important diterpenoids associated with vision are those with vitamin A activity. Besides this, retinoids (e.g.retinol, 29) influence the growth and activate the tumor suppressor genes [25, 32]. Gibberellic acid (30), a plant hormone controlling plant growth and development is a tetracyclic diterpene acid [33]. Carnosic acid (31) and carnosol (32) are found in the herb rosemary (Rosmarinus officinalis). Carnosol (32) is an ortho-diphenolic diterpenoid possessing anti oxidant, anti inflammatory and anti cancer activity [34]. Carnosic acid (31) shows

antimicrobial and antioxidative properties and it is also used as preservatives [35]. Structures of some important diterpenoids are given in Figure 1.9.

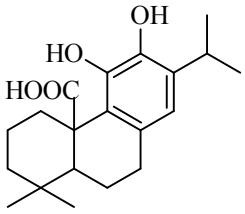
The main group of tetraterpenoids is the carotenoids which are found in plants as well as in humans. Photoprotection, light harvesting in photosynthesis and pigmentation are the main functions of carotenoids in plants [36]. For man, their main function is associated with vision. Carotenoids are good anti-oxidizing agents. They are involved in scavenging of singlet oxygen and peroxide radical and thereby protect human from various disease like cancer [37]. Some important carotenoids are lycopene (33), β-carotene (34), lutein (35) etc are shown in Figure 1.10.



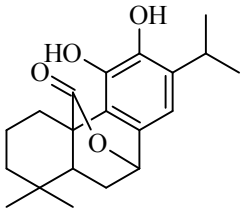
Retinol (29)



Gibberellic acid (30)

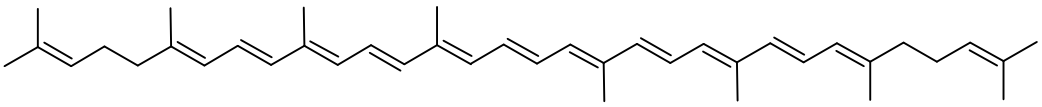


Carnosic acid (31)

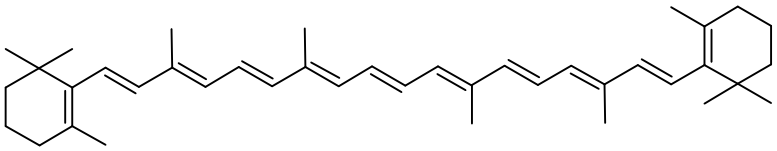


Carnosol (32)

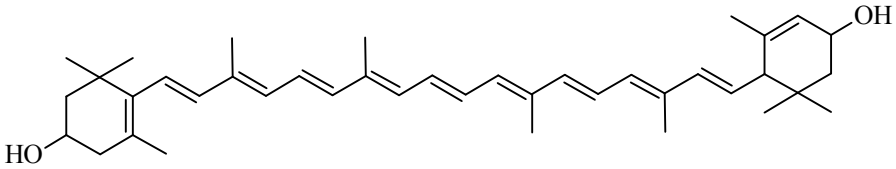
Figure 1.9. Structure of some important diterpenoids



Lycopene (33)



β-Carotene (34)



Lutein (35)

Figure 1.10. Structure of some important carotenoids

1.5.6. Therapeutically used terpenoids

Terpenoids are used for the treatment of various diseases [38]. Table 1.2 depicted a few of them.

Table 1.2. Terpenoids approved for therapeutic use

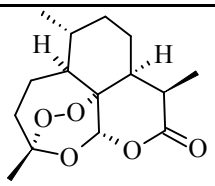
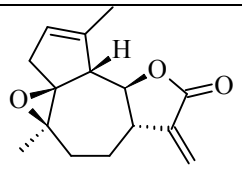
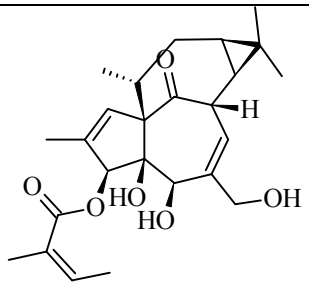
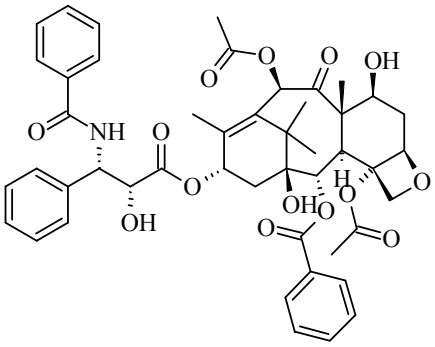
Generic name (trade name)	Chemical structure	Plant species	Type of terpenoids	Indication and mechanism of action
Artemisinin (Artemisin)		*Artemisia annua L.*	Sesquiterpene lactone endoperoxide	Malaria treatment (radical formation)

Table 1.2 (continued)

Generic name (trade name)	Chemical structure	Plant species	Type of terpenoids	Indication and mechanism of action
Arglabin (Arglabin)		*Artemisia obtusiloba var. glabra Ledeb.*	Sesquiterpene lactone	Cancer chemotherapy (inhibition of farnesyl transferase)
Ingenol mebutate (Picato)		*E. peplus L.*	Diterpenoid	Actinic keratosis (inducer of cell death)
Paclitaxel (Nanoxel)		*T. brevifolia Nutt.*	Diterpenoid core	Cancer chemotherapy (antimitotic agent)

1.5.7. Important receptors of terpenoids

1.5.7.1. Glycogen phosphorylase

Glycogen is the principal reserve of carbohydrate in animals and its breakdown into glucose-1-phosphate is mediated by glycogen phosphorylase (GP) with the help of a debranching enzyme [39]. The brain, liver, and muscle isoforms of GP share approximately 80% sequence homology and each has an important role in glycogen metabolism. The brain isoform supplies glucose during the period of insufficient glucose level in brain. The muscle isoform gives energy for muscle contraction while the liver isoform provides glucose to the rest of the body and hence it is an attractive target for treatment of type 2 diabetes [40-43].

GP exists in two forms: GPa and GPb and they are interconvertible. GPa has high activity and remains predominantly in the active R state (Relaxed state) while GPb has low activity and resides mainly in the T state (Tense state). During physical activity or hormone simulation, the enzyme is converted from T state b form (GPb) to the R state a form (GPa) through the phosphorylation of Ser14 which is catalyzed by phosphorylase kinase [44, 45]. The regulatory sites of GP are: glucose analogues at the catalytic site, azasugar inhibitors, lactones at the allosteric site (AMP), caffeine at the purine inhibitor site, indole-2-carboxamide at the indole binding site and cyclodextrins at the glycogen storage site [46, 47]. The X-ray analysis suggests that pentacyclic triterpenes bind at the allosteric site [48].

1.5.7.2. Protein tyrosine phosphatase 1B

Reversible Protein tyrosine phosphorylation is important for nearly all cellular processes such as growth, differentiation, migration, survival, and apoptosis. Protein tyrosine kinases (PTKs) are enzymes that catalyze protein tyrosine phosphorylation where as protein-tyrosine phosphatases

(PTPs) catalyze the dephosphorylation process [49]. In men, more than one hundred PTPs are present and they function as negative or positive modulators of biological pathways [50]. Among the member of PTPs, protein tyrosine phosphatase 1B (PTP1B) negatively regulates insulin signaling pathway and is an excellent drug targets for type 2 diabetes and insulin resistance [51, 52].

A number of natural compounds show PTP1B inhibitory activity. Thareja et al. reviewed approximately 50 natural PTP1B inhibitors [53]. The first reported sesterterpenoid which shows PTP1B inhibitory activity is sulfircin 236 isolated as its N,N-dimethylguanidinium salt from a deep-water sponge Ircinia (unknown species) collected from Andros and Bahamas [54]. In 2002, five natural flavonoids were reported as PTP1B inhibitors [55]. Since then, approximately 300 known or new natural compounds have been identified which possess PTP1B inhibitory activity.

PTP1B consists of 435 amino acids with active site (site A) of approximately 8 to 9 Å depth and is defined by residues His-Cys-Ser-Ala-Gly-Ile-Gly-Arg (214 to 221). The important amino acid residues of second binding site (site B) are Arg24, Arg254, and Glu262. Other amino acid residues in site B are Tyr46, Asp48, Val49, Ile219, and Met258. Molecular docking studies indicate that triterpenes bind in the site B, not in the active site [56, 57].

1.5.7.3. Lipoxygenase

Lipoxygenases are a class of non-heme iron containing oxidative enzymes, occurring in a number of plants and animals [58, 59]. These enzymes catalyze the incorporation of molecular oxygen in the naturally occurring poly-unsaturated fatty acids (PUFAs) such as arachidonic acid and linoleic acid [60]. More importantly, lipoxygenases are involved in the regulation of inflammatory responses that can promote human disease. For example, human 5-lipoxygenase

(5-HLO), human 12-lipoxygenase (12-HLO) and human 15- lipoxygenase (15-HLO) are involved in several diseases like asthma, arthritis, allergy, psoriasis, atherosclerosis and tumorigenesis [61-66].

Several studies have suggested that diets containing high fat, particularly omega-6 polyunsaturated fatty acids, are linked with pancreatic cancer development and growth [67, 68]. The pathways associated in the conversion of unsaturated fatty acid arachidonic acid and linoleic acid to their lipid metabolites such as prostaglandins and leukotrienes which are involved in development and growth of multiple human cancers [69, 70]. Hence, the blockade of lipoxygenase pathway inhibits pancreatic cancer cell proliferation and induces apoptosis through the mitochondria-mediated pathway, with cytochrome c release and caspase activation [71].

The lipoxygenase inhibitors are classified into two groups, redox and nonredox inhibition. The redox active compound reduces lipoxygenase from ferric oxidation state to its inactive ferrous form where as allosteric inhibition can occur in nonredox mechanism [72, 73].

1.6. Quantitative structure-activity relationship

A number of natural products were isolated and attempts were made to correlate their chemical structures with their physiological activities. Though the physiological activity of a compound is associated with a particular structural unit or group, there are no hard and fast rules connecting chemical structure and physiological activity. Quantitative structure-activity relationship (QSAR) makes it feasible to predict the activities of a given compound as a function of its molecular substituent. New compounds with similar structural features can also be incorporated in the development of QSAR models assuming that they possess similar activities. Hence QSAR methods have become major tools in modelling and designing novel compounds.

1.6.1. Historical development of QSAR

More than a century ago, in 1863, Cros showed a relationship between the toxicity of primary aliphatic alcohols and their water solubility [74]. In 1868, Crum-Brown and Fraser formulated a suggestion that the physiological action of a substance is a function of its chemical composition and constitution [75]. Shortly after, Richet (1893), Meyer (1899) and Overton (1901) separately concluded that narcotic (depressant) activity is dependent on the lipophilicity of the molecules [76-78]. In 1935 Hammett proposed a relationship which deals with the electron donating and withdrawing substituents m- and p- to the reaction site in monosubstituted benzene [79, 80]. The mathematical expression of Hammett equation is:

$$\log (K/K_0) = \sigma\rho \quad \ldots\ldots\ldots \quad (1.1)$$

Where K and K_0 are the equilibrium constant of substituted and unsubstituted phenyl compounds of a given reaction. The σ is a substituent constant and ρ is a reaction constant. Taft (1952, 1953) has examined aliphatic reactions and incorporates a steric substituent parameter, Es [81]. The contributions from Hammett and Taft set forth the mechanistic basis for the development of the QSAR paradigm by Hansch and Fujita (1964). The mathematical combination of Hammett σ constants and log P values (P is the octanol-water partition coefficient of the unionized molecule) gives the linear Hansch equation and its many extended forms [82, 83].

$$\log (1/C) = k_1\pi + k_2\sigma + k_3 \quad \ldots\ldots\ldots \quad (1.2)$$

Where C is the molar concentration, the constants k_1, k_2, k_3 are obtained via least square methods and $\pi = \log Px - \log P_H$. P_H and Px are the partition coefficient of the parent molecule and its derivative. This equation is better than Hammett equation. However in case of highly hydrophilic molecules, Hansch postulated parabolic equation:

$$\log (1/C) = -k_1(\log P)^2 + k_2(\log P) + k_3\sigma + k_4 \ldots\ldots\ldots (1.3)$$

Free and Wilson constructed a model of substituent contribution to the overall biological activity [84]. It is given by the following equation:

$$BA = \Sigma a_i x_i + u \ldots\ldots\ldots (1.4)$$

where BA is the biological activity, u is the average contribution of the parent molecule, and a_i is the contribution of each structural feature; x_i denotes the presence $x_i = 1$ or absence $x_i = 0$ for a particular structural fragment. In 1971, Fujita and Ban simplified the Free and Wilson equation by using logarithm of activity which brought the activity parameter in line with other free energy-related terms [85]. In the 1970s, quantum chemical indices appear in QSAR modeling [86]. Topological descriptors are actually structural descriptors as they are only based on the two-dimensional chemical structure. These descriptors, which were originally proposed by Balaban [87], Randić [88], Kier and Hall [89], provided significant developments of the QSAR approaches in the mid-1980s. However simple descriptors like counts of atoms/fragments or topological descriptors have only a limited utility due to the lack of consideration of the 3D structure of the molecules, leading to the development of the 3D-QSAR. A large variety of geometrical descriptors like shadow indexes [90], charged partial surface area descriptors [91], WHIM descriptors [92], gravitational indexes [93], EVA descriptors [94], 3D-MoRSE descriptors [95], GETAWAY descriptors [96] etc which were derived from the 3D geometrical structure of a molecule are now used in QSAR modeling. In 1988, Cramer et al. proposed a powerful 3D-QSAR methodology, Comparative Molecular Field Analysis (CoMFA) [97]. In 3D-QSAR method the biological activity of a ligand can be predicted from the molecular interaction field properties in 3D space, such as steric demand, lipophilicity, and electrostatic interactions.

Finally, the scientific community has shown interest in virtual screening and moleculer design, for which several similarity/diversity approaches, cell-based methods, and scoring functions have been developed on the basis of the substructural descriptors such as molecular fingerprints [98, 99].

1.6.2. Development of QSAR model

The construction of QSAR model consists of two main steps: (i) selection of molecules (data set) followed by determination of molecular descriptors. Low quality descriptors are excluded as they will lower the quality of the model. Hence, it is better to use the most informative descriptors in the QSAR modeling. However in many cases too much information in the independent variables (the descriptors) with respect to the response is often seen as noise in the model, thus giving instable or unpredictive models. (ii) Modeling methods like multiple linear regression, logistic regression and machine learning methods etc are used for correlating molecular descriptors with observed activities. During the development of the QSAR model the data set are divided into two groups: training set and test set. The descriptors of the training set are used to build QSAR models that describe the empirical relationship between the structure and activity. The finalized QSAR models are verified by the statistical evaluation and with a testing set. Figure 1.11 illustrates general overview of developing QSAR process.

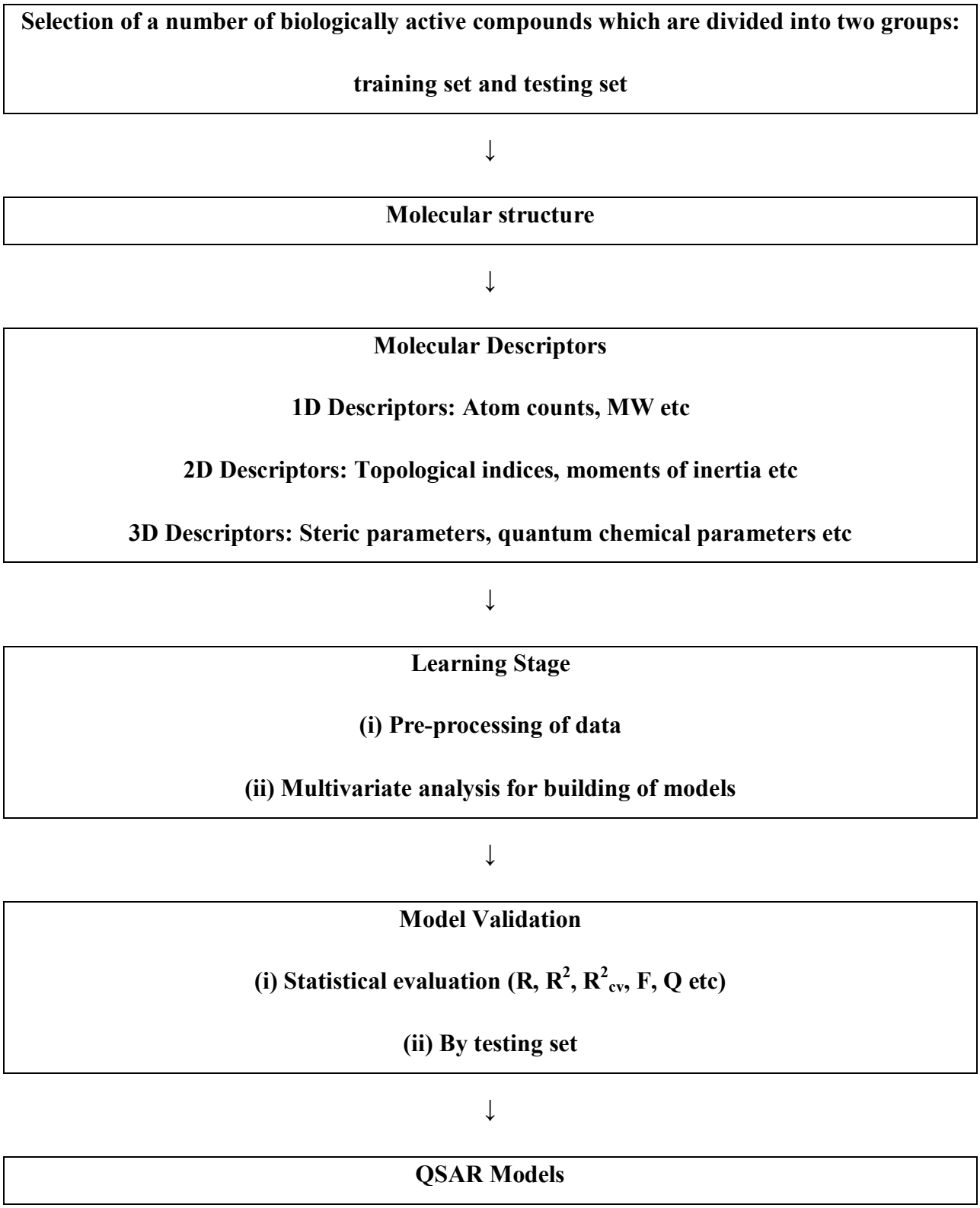


Figure 1.11. General overview of developing QSAR process

1.6.3. Conceptual DFT approach in QSAR analysis

In recent years, QSAR studies have been carried out using conceptual density functional theory (DFT) based descriptors. These descriptors are able to provide accurate quantitative description of the molecular structures and chemical properties. The important DFT descriptors which have found immense usefulness in QSAR studies are molecular orbital energies, chemical hardness, chemical softness, dipole moments etc.

The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies are important quantum chemical descriptors. These orbitals play a major role in many chemical reactions by determining electronic band gaps in solids and are also responsible for the formation of many charge transfer complexes [100, 101]. According to the frontier molecular orbital theory, the formation of a transition state is due to an interaction between HOMO and LUMO of the reacting species [102]. The HOMO and LUMO energies are also important in radical reactions [103, 104]. Ionization energy measures the energy of removing an electron from the HOMO and characterizes the susceptibility of the molecule toward attack by electrophiles while electron affinity represents the energy liberated when an electron is brought from infinity to the LUMO and characterizes the susceptibility of the molecule toward attack by nucleophiles. The hard and soft acid base concept of nucleophiles and electrophiles are directly related to the relative energy of the HOMO/LUMO orbitals [105]. The HOMO/LUMO gap energy is an important stability index. A large HOMO/LUMO separation implies high stability for the molecule in chemical reactions. The HOMO/LUMO gap energy also estimates the lowest excitation energy of the molecule [106]. The often used polarity quantity of the molecule is the dipole moment which reflects the global polarity of a molecule [107].

1.7. Objectives of the thesis

i) Construction of QSAR equations between molecular properties and the biological activities of a number of terpenoids and the quality of the model can be assessed by several statistical parameters.

ii) Docking study was performed to explore the binding mode of different bioactive terpenoids.

iii) To study the redox potential of bioactive terpenoids.

iv) To study the structure of bioactive terpenoids by quantum chemical methods and compared it with the experimental value.

Every chapter in this thesis is complete by itself; that is, it contains its own introduction, complete list of references, figures, tables, and interim conclusions etc.

1.8. References

[1] G.M. Cragg, D.J. Newman, Biodiversity: A continuing source of novel drug leads, Pure Appl Chem. 77 (2005) 7-24.

[2] H.F. Ji, X.J. Li, H.Y. Zhang, Natural products and drug discovery, EMBO rep. 10 (2009) 194-200.

[3] D.J. Newman, Natural products as leads to potential drugs: an old process or the new hope for drug discovery?, J Med Chem. 51 (2008) 2589-2599.

[4] W.J. Zhang, G.H. Liu, Coevolution: A synergy in biology and ecology, Selforganizology. 2 (2015) 35-38.

[5] S.J. Cutler, H.G. Cutler, Biologically Active Natural Products: Pharmaceuticals, CRC Press, New York, 2000.

[6] B. delasHeras, B. Rodríguez, L. Boscá, A.M. Villar, Terpenoids: sources, structure elucidation and therapeutic potential in inflammation, Curr Top Med Chem. 3 (2003) 171-185

[7] D. Tholl, Biosynthesis and biological functions of terpenoids in plants, Adv Biochem Eng Biotechnol. 148 (2015) 63-106.

[8] Z.L. Ruzicka, The isoprene rule and the biogenesis of terpenic compounds, Experientia. 9 (1953**)** 357-367.

[9] J. Gershenzon, W. Kreis, Biosynthesis of monoterpenes, sesquiterpenes, diterpenes, sterols, cardiac glycosides and steroid saponins, Biochemistry of plant secondary metabolites,

Annual plant reviews, in: M. Wink (Ed), Sheffield Academic Press: Sheffield, UK, Vol. 2, 1999.

[10] J. Gershenzon, N. Dudareva, The function of terpene natural products in the natural world, Nat Chem Biol. 3 (2007) 408-414.

[11] M. Rohmer, The Discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants, Nat Prod Rep. 16 (1999) 565-574.

[12] I.L Finar, Stereochemistry and the chemistry of natural products, Pearson Education, Singapore, Vol. 2, 2004.

[13] P. Crabbé, Ord and Cd in chemistry and biochemistry, Academic Press, London, 1972.

[14] N. Harada, K. Nakanishi, Circular dichroic spectroscopy, Exciton coupling in organic stereochemistry, University Science Books, Mill Valley, CA, 1983.

[15] E.L. Eliel, S.H. Wilen, Stereochemistry of organic compounds, Wiley-Interscience, New York, 1994.

[16] A.A. Saddiq, S.A. Khayyat, Chemical and antimicrobial studies of monoterpene: Citral, Pestic Biochem Physiol. 98 (2010) 89-93.

[17] C. de Bona da Silva, S.S. Guterres, V. Weisheimer, E.E.S. Schapoval, Antifungal activity of the lemongrass Oil and citral against Candida spp., Braz J Infect Dis. 12 (2008) 63-66.

[18] K. Ghosh, Anticancer effect of lemongrass oil and citral on cervical cancer cell lines, Phcog Commn. 3 (2013) 41-48.

[19] W. Chaouki, D.Y. Leger, B. Liagre, J.L. Beneytout, M. Hmamouchi, Citral inhibits cell proliferation and induces apoptosis and cell cycle arrest in MCF-7 cells, Fundam Clin Pharmacol. 23 (2009) 549-556.

[20] H. Xia, W. Liang, Q. Song, X. Chen, X. Chen, J. Hong, The in vitro study of apoptosis in NB4 cell induced by citral, Cytotechnology 65 (2013) 49-57.

[21] S. Zeng, A. Kapur, M.S. Patankar, M.P. Xiong, Formulation, characterization, and antitumor properties of trans- and cis-citral in the 4T1 breast cancer xenograft mouse model, Pharm Res. 32 (2015) 2548-2558.

[22] P.L. Crowell, Monoterpenes in breast cancer chemoprevention, Breast Cancer Res Treat. 46 (1997) 191-197.

[23] P.L. Crowell, Prevention and therapy of cancer by dietary monoterpenes, J Nutr. 129 (1999) 775S-778S.

[24] M.N. Gould, Cancer chemoprevention and therapy by monoterpenes, Environ Health Perspect. 105 (1997) 977-979.

[25] K.H. Wagner, I. Elmadfa, Biological relevance of terpenoids-overview focusing on mono, di and tetraterpenes, Ann Nutr Metab. 47 (2003) 95-106.

[26] H.S. Choi, H.S. Song, H. Ukeda, M. Sawamura, Radical scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl, J Agric Food Chem. 48 (2000) 4156-4161.

[27] L. Caputi, E. Aprea, Use of terpenoids as natural flavouring compounds in food industry, Recent Pat Food Nutr Agric. 3 (2011) 9-16.

[28] J.H. Joo, A.M. Jetten, Molecular mechanisms involved in farnesol-induced apoptosis, Cancer Lett. 287 (2010) 123-135.

[29] L. Kromidas, E. Perrier, J. Flanagan, R. Rivero, I. Bonnet, Release of antimicrobial actives from microcapsules by the action of axillary bacteria, Int J Cosmet Sci. 28 (2006) 103-108.

[30] A. Bahi, S.A Mansouri, E.A Memari, M.A Ameri, S.M Nurulain, S. Ojha, β-Caryophyllene, a CB_2 receptor agonist produces multiple change relevant toanxiety and depression in mice, Physiol Behav. 135 (2014) 119-124.

[31] M. Seo, T. Koshiba, Complex regulation of ABA biosynthesis in plants, trends in plant sci. 7 (2002) 41-48.

[32] T.R. Evans, S.B. Kaye, Retinoids: present role and future potential, Br J Cancer. 80 (1999) 1-8.

[33] R. Gupta, S.K. Chakrabarty, Gibberellic acid in plant Still a mystery unresolved, Plant Signal Behav. 8 (2013) e25504.

[34] J.J. Johnson, Carnosol: a promising anti-cancer and anti-inflammatory agent, Cancer Lett. 305 (2011) 1-7.

[35] S. Birtić, P. Dussort, F.X. Pierre, A.C. Bily, M. Roller, Carnosic acid, Phytochemistry. 115 (2015) 9-19.

[36] G.E. Bartley, P.A. Scolnik, Plant carotenoids: pigments for photoprotection, visual attraction, and human health, Plant Cell. 7 (1995) 1027-1038.

[37] J. Grassmann, Terpenoids as plant antioxidants, Vitam Horm. 72 (2005) 505-535.

[38] A.G. Atanasov, B. Waltenberger, E.M.P. Wenzig , T. Linder , C. Wawrosch, P. Uhrin, V. Temml, L. Wang, S. Schwaiger, E. Heiss, J.M. Rollinger, D. Schuster, J.M. Breuss, V. Bochkov, M.D. Mihovilovic, B. Kopp, R. Bauer, V.M. Dirsch, H. Stuppner, Discovery and resupply of pharmacologically active plant-derived natural products: a review, Biotechnol Adv. 33 (2015) 1582-1614.

[39] M. Bollen, S. Keppens, W. Stalmans, Specific features of glycogen metabolism in the liver, Biochem J. 336 (1998) 19-31.

[40] H.G. Hers, The control of glycogen metabolism in the liver, Annu Rev Biochem. 45 (1976) 167-190.

[41] W. Stalmans, M. Laloux, H.G. Hers, The interaction of liver phosphorylase a with glucose and AMP, Eur J Biochem. 49 (1974) 415-427.

[42] W. Pimenta, N. Nurjhan, P.A. Jansson, M. Stumvoll, J. Gerich, M. Korytkowski, Glycogen: its mode of formation and contribution to hepatic glucose output in postabsorptive humans, Diabetologia. 37 (1994) 697-702.

[43] M.K. Hellerstein, R.A. Neese, P. Linfoot, M. Christiannsen, S. Turner, A. Letscher, Hepatic gluconeogenic fluxes and glycogen turnover during fasting in humans. A stable isotope study, J Clin Invest. 100 (1997) 1305-1319.

[44] N.G. Oikonomakos, E.D. Chrysina, M.N. Kosmopoulou, D.D. Leonidas, Crystal structure of rabbit muscle glycogen phosphorylase a in complex with a potential hypoglycaemic drug at 2.0 Å resolution, Biochim Biophys Acta. 1647 (2003) 325-332.

[45] V.L. Rath, M. Ammirati, D.E. Danley, J.L. Ekstrom, E.M. Gibbs, T.R. Hynes, A.M. Mathiowetz, R.K. McPherson, T.V. Olson, J.L. Treadway, D.J. Hoover, Human liver glycogen phosphorylase inhibitors bind at a new allosteric site, Chem Biol. 7 (2000) 677-682.

[46] R. Kurukulasuriya, J.T. Link, D.J. Madar, Z. Pei, S.J. Richards, J.J. Rohde, A.J. Souers, B.G. Szczepankiewicz, Potential drug targets and progress towards pharmacologic inhibition of hepatic glucose production, Curr Med Chem. 10 (2003) 123-153.

[47] Z. Liang, L. Zhang, L. Li, J. Liu, H. Li, L. Zhang, L. Chen, K. Cheng, M. Zheng, X. Wen, P. Zhang, J. Hao, Y. Gong, X. Zhang, X. Zhu, J. Chen, H. Liu, H. Jiang, C. Luo, H. Sun, Identification of pentacyclic triterpenes derivatives as potent inhibitors against glycogen phosphorylase based on 3D-QSAR studies, Eur J Med Chem. 46 (2011) 2011-2021.

[48] X. Wen, H. Sun, J. Liu, K. Cheng, P. Zhang, L. Zhang, J. Hao, L. Zhang, P. Ni, S.E. Zographos, D.D. Leonidas, K.M. Alexacou, T. Gimisis, J.M. Hayes, N.G. Oikonomakos, Naturally occurring pentacyclic triterpenes as inhibitors of glycogen phosphorylase: synthesis, structure-activity relationships, and X-ray crystallographic studies, J Med Chem. 51 (2008) 3540-3554.

[49] T. Hunter, Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling, Cell. 80 (1995) 225-236.

[50] A. Alonso, J. Sasin, N. Bottini, I. Friedberg, I. Friedberg, A. Osterman, A. Godzik, T. Hunter, J. Dixon, T. Mustelin, Protein tyrosine phosphatases in the human genome, Cell. 117 (2004) 699-711.

[51] Z.Y. Zhang, S.Y. Lee, PTP1B inhibitors as potential therapeutics in the treatment of type 2 diabetes and obesity, Expert Opin Investig Drugs. 12 (2003) 223-233.

[52] A.P. Combs, Recent advances in the discovery of competitive protein tyrosine phosphatase 1B inhibitors for the treatment of diabetes, obesity, and cancer, J Med Chem. 53 (2010) 2333-2344.

[53] S. Thareja, S. Aggarwal, T.R. Bhardwaj, M. Kumar, Protein tyrosine phosphatase 1B inhibitors: a molecular level legitimate approach for the management of diabetes mellitus, Med Res Rev. 32 (2012) 459-517.

[54] R.E. Cebula, J.L. Blanchard, M.D. Boisclair, K. Pal, N.J. Bockovich, Synthesis and phosphatase inhibitory activity of analogs of sulfircin, Bioorg Med Chem Lett. 7 (1997) 2015-2020.

[55] R.M. Chen, L.H. Hu, T.Y. An, J. Li, Q. Shen, Natural PTP1B inhibitors from Broussonetia papyrifera, Bioorg Med Chem Lett. 12 (2002) 3387-3390.

[56] C.S. Jiang, L.F. Liang, Y.W. Guo, Natural products possessing protein tyrosine phosphatase 1B (PTP1B) inhibitory activity found in the last decades, Acta Pharmacologica Sinica. 33 (2012) 1217-1245.

[57] J.J. Ramírez-Espinosa, M.Y. Rios, S.L. Martínez, F.L. Vallejo, J.L. Medina-Franco, P. Paoli, G. Camici, G. Navarrete-Vázquez, R. Ortiz-Andrade, S. Estrada-Soto, Antidiabetic activity of some pentacyclic acid triterpenoids, role of PTP-1B: in vitro, in silico, and in vivo approaches, Eur J Med Chem. 46 (2011) 2243-2251.

[58] E.I. Solomon, J. Zhou, F. Neese, E.G. Pavel, New insights from spectroscopy into the structure/function relationships of lipoxygenases, Chem Biol. 4 (1997) 795-808.

[59] H.W. Gardner, Biological roles and biochemistry of the lipoxygenase pathway, Hortscience. 30 (1995) 197-205.

[60] R. Wisastra, F.J. Dekker, Inflammation, cancer and oxidative lipoxygenase activity are intimately linked, Cancers. 6 (2014) 1500-1521.

[61] L.A. Dailey, P. Imming, 12-Lipoxygenase: classification, possible therapeutic benefits from inhibition, and inhibitors, Curr Med Chem. 6 (1999) 389-398.

[62] V.E. Steele, C.A. Holmes, E.T. Hawk, L. Kopelovich, R.A. Lubet, J.A. Crowell, C.C. Sigman, G.J. Kelloff, Lipoxygenase inhibitors as potential cancer chemopreventives, Cancer Epidem Biomar. 8 (1999) 467-483.

[63] B. Samuelsson, S.E. Dahlen, J.A. Lindgren, C.A. Rouzer, C.N. Serhan, Leukotrienes and lipoxins: structures, biosynthesis, and biological effects, Science. 237 (1987) 1171-1176.

[64] X.Z. Ding, W.G. Tong, T.E. Adrian, 12-Lipoxygenase metabolite 12(S)-HETE stimulates human pancreatic cancer cell proliferation via protein tyrosine phosphorylation and ERK activation, Int J Cancer. 94 (2001) 630-636.

[65] J.A. Cornicelli, B.K. Trivedi, 15-Lipoxygenase and its inhibition: a novel therapeutic target for vascular disease, Curr Pharm Des. 5 (1999) 11-20.

[66] U.P. Kelavkar, J.B. Nixon, C. Cohen, D. Dillehay, T.E. Eling, K.F. Badr, Overexpression of 15-lipoxygenase-1 in PC-3 human prostate cancer cells increases tumorigenesis, Carcinogenesis. 22 (2001) 1765-1773.

[67] B.D. Roebuck, D.S. Longnecker, K.J. Baumgartner, C.D. Thron, Carcinogen-induced lesions in the rat pancreas: effects of varying levels of essential fatty acid, Cancer Res. 45 (1985) 5252-5256.

[68] J. Zhang, V.L. Go, High fat diet, lipid peroxidation, and pancreatic carcinogenesis, Adv Exp Med Biol. 399 (1996) 165-172.

[69] D.P. Rose, Dietary fatty acids and cancer, Am J Clin Nutr. 66 (1997) 998S-1003S.

[70] P.L. Zock, M.B. Katan, Linoleic acid intake and cancer risk: a review and meta-analysis, Am J Clin Nutr. 68 (1998) 142-153.

[71] W.G. Tong, X.Z. Ding, R.C. Witt, T.E. Adrian, Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway, Mol Cancer Therapeutics. 1 (2002) 929-935.

[72] C. Kemal, P. Louis-Flamberg, R. Krupinski-Olsen, A.L. Shorter, Reductive inactivation soybean lipoxygenase-1 by catechols: a possible mechanism for regulation of lipoxygenase activity, Biochemistry. 26 (1987) 7064-7072.

[73] R. Mogul, E. Johansen, T.R. Holman, Oleyl sulfate reveals allosteric inhibition of soybean lipoxygenase-1 and human 15-lipoxygenase, Biochemistry. 39 (2000) 4801-4807.

[74] A.F.A. Cros, Ph.D. dissertation thesis: Action de l'alcohol amylique sur l'organisme, University of Strasbourg, Strasbourg, 1863.

[75] A. Crum-Brown, T.R. Fraser, On the connection between chemical constitution and physiological action. Part 1. On the physiological action of the ammonium bases, derived

from strychia, brucia, thebaia, codeia, morphia and nicotia, Trans R Soc Edinburgh. 25 (1868) 151-203.

[76] C. Richet, On the relationship between the toxicity and the physical properties of substances, Compt Rendus Seances Soc Biol. 9 (1893) 775-776.

[77] E. Overton, Osmotic properties of cells in the bearing on toxicology and pharmacology, Z Physik Chem. 22 (1897) 189-209.

[78] H. Meyer, On the theory of alcohol narcosis I. Which property of anesthetics gives them their narcotic activity?, Arch Exp Pathol Pharmakol. 42 (1899) 109-118.

[79] L.P. Hammett, Some relations between reaction rates and equilibrium constants, Chem Rev. 17 (1935) 125-136.

[80] L.P. Hammett, The effect of structure upon the reactions of organic compounds. benzene derivatives, J Am Chem Soc. 59 (1937) 96-103.

[81] R.W. Taft, Polar and steric substituent constants for aliphatic and o- Benzoate groups from rates of esterification and hydrolysis of esters1, J Am Chem Soc. 74 (1952) 3120-3128.

[82] C. Hansch, T. Fujita, p-σ-π Analysis. A method for the correlation of biological activity and chemical structure, J Am Chem Soc. 86 (1964) 1616-1626.

[83] C. Hansch, Quantitative approach to biochemical structure-activity relationships, Acc Chem Res. 2 (1969) 232-239.

[84] S.M. Free Jr, J.W. Wilson, A Mathematical contribution to structure-activity studies, J Med Chem. 7 (1964) 395-399.

[85] T. Fujita, T. Ban, Structure-activity study of phenethylamines as substrates of biosynthetic enzymes of sympathetic transmitters, J Med Chem. 14 (1971) 148-152.

[86] L.B. Kier, Molecular orbital theory in drug research, Academic Press, New York, 1971.

[87] A.T. Balaban, F. Harary, The characteristic polynomial does not uniquely determine the topology of a molecule, J Chem Doc. 11 (1971) 258-259.

[88] M. Randić, On the recognition of identical graphs representing molecular topology, J Chem Phys. 60 (1974) 3920-3928.

[89] L.B. Kier, L.H. Hall, W.J. Murray, M. Randic, Molecular connectivity I: relationship to nonspecific local anesthesia, J Pharm Sci. 64 (1975) 1971-1974.

[90] R.H. Rohrbaugh, P.C. Jurs, Descriptions of molecular shape applied in studies of structure/activity and structure/property relationships, Anal Chim Acta. 199 (1987) 99-109.

[91] D.T. Stanton, P.C. Jurs, Development and use of charged partial surface area structural descriptors in computer-assisted quantitative structure-property relationship studies, Anal Chem. 62 (1990) 2323-2329.

[92] R. Todeschini, M. Lasagni, E. Marengo, New molecular descriptors for 2D- and 3D-structures, Theory J Chemom. 8 (1994) 263-273.

[93] A.R. Katritzky, L. Mu, V.S. Lobanov, M. Karelson, Correlation of boiling points with molecular structure. 1. A training set of 298 diverse organics and a test set of 9 simple inorganics, J Phys Chem. 100 (1996) 10400-10407.

[94] A.M. Ferguson, T.W. Heritage, P. Jonathon, S.E. Pack, L. Phillips, J. Rogan, P.J. Snaith, EVA: a new theoretically based molecular descriptor for use in QSAR/QSPR analysis, J Comput Aided Mol Des. 11 (1997) 143-152.

[95] J. Schuur, P. Selzer, J. Gasteiger, The coding of the three-dimensional structure of molecules by molecular transforms and its application to structure-spectra correlations and studies of biological activity, J Chem Inf Comput Sci. 36 (1996) 334-344.

[96] V. Consonni, R. Todeschini, M. Pavan, Structure/response correlations and similarity/diversity analysis by GETAWAY descriptors. Part 1. Theory of the novel 3D molecular descriptors, J Chem Inf Comput Sci. 42 (2002) 682-692.

[97] R.D. Cramer, D.E. Patterson, J.D. Bunce, Comparative molecular field analysis (CoMFA). 1. Effect of shape on binding of steroids to carrier proteins, J Am Chem Soc. 110 (1988) 5959-5967.

[98] J. Gasteiger, Handbook of chemoinformatics. From data to knowledge in 4 volumes, Wiley-VCH, Weinheim, 2003.

[99] T.I. Oprea, 3D QSAR modeling in drug design, in: P. Bultinck, H. De Winter, W. Langenaeker, J.P. Tollenaere (Eds.), Computational medicinal chemistry for drug discovery, Marcel Dekker, New York, 2004.

[100] R. Franke, Theoretical Drug Design Methods, Elsevier, Amsterdam, 1984.

[101] K. Osmialowski, J. Halkiewicz, A. Radecki, R. Kaliszan, Quantum chemical parameters in correlation analysis of gas-liquid chromatographic retention indices of amines, J Chromatogr. 346 (1985) 53-60.

[102] K. Fukui, Theory of orientation and stereoselection, Springer- Verlag, New York, 1975.

[103] H. Sklenar, J. Jäger, Molecular structure-biological activity relationships on the basis of quantum-chemical calculations, Int J Quant Chem. 16 (1979) 467-484.

[104] K. Tuppurainen, S. Lötjönen, R. Laatikainen, T. Vartiainen, U. Maran, M. Strandberg, T. Tamm, About the mutagenicity of chlorine-substituted furanones and halopropenals. A QSAR study using molecular orbital indices, Mutat Res Fund Mol Mech Mut. 247 (1991) 97-102.

[105] I. Fleming, Frontier orbitals and organic chemical reactions, John Wiley & Sons, New York, 1976.

[106] D.F.V. Lewis, C. Ioannides, D.V. Parke, Interaction of a series of nitriles with the alcohol inducible isoform of P450- computer analysis of structure-activity relationships, Xenobiotica. 24 (1994) 401-408.

[107] M. Karelson, V.S. Lobanov, Quantum-Chemical Descriptors in QSAR/QSPR Studies, Chem Rev. 96 (1996) 1027-1043.