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Biotransformation of Pharmaceutical Drugs by Plant Tissue Culture: Challenges and New Opportunities

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Abstract

Biotransformation of pharmaceutical drugs utilizing tissue cell culture is still new unexplored area of scientific research. It holds great potential in terms of modifying available drugs and natural products aiming to improve their therapeutic activity and reduce any adverse toxic effects. The use of microorganisms (bacteria and fungi) in the biotransformation of pharmaceutical drugs has been practiced by scientists over the last five decades. Significant achievements in the field of synthesis and modification of pharmaceutical drugs have been reported. The main advantage of utilizing biotransformation for the synthesis of pharmaceutical drugs is eliminating the need for lengthy tedious synthetic procedures utilizing toxic reagents and catalysts. Biotransformation utilizes only benign solvents (e.g. water, ethanol), enzymes as catalysts under normal temperature eliminating the production of toxic waste materials.

Keywords: Biotransformation, Taxol, Atorvastatin, Loratadine, Desloratadine, Plant tissue culture.

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1. INTRODUCTION

The use of enzymes from microorganisms and plant tissue cultures for pharmaceutical drugs and natural products modification is undoubtedly an ideal choice toward "greening" chemical reactions. Biotransformation techniques have been known since the early stages of human civilization and have been used since then to make fermented foods and beverages (e.g., beer preparation by ancient Egyptians). However, biotransformation started to be an important technique for the pharmaceutical industry with modification of the steroid nucleus emerged in the fifties of the last century. The industrial applications of biotransformation in food processing and in the production of advanced chemicals, drugs, and detergents have been undergoing a drastic development in the last decades. The need for a synthesis of pure chiral drugs and the ease of applying biotransformation techniques for their preparation has attracted more attention to this field of science. The chiral drug market is estimated to grow from 25% up to 70% of the total pharmaceutical drug market by the first half of the 21st century. These expectations show the importance of biotransformation and biocatalysis to the pharmaceutical industry [1, 42, 43, 44, 45, 46, 47].

The use of plant tissue culture for the biotransformation of pharmaceutical drugs is a newly emerged area of biotechnology. It holds the potential of forming new pharmaceutical entities from existing drug entities having novel pharmacological activity. This technique depends on the ability of plant cell culture to catalyze the transformation of a readily available or inexpensive precursor into a more valuable final product. So, plant tissue culture focuses on the conversion of a small part of the molecule (functional group modification) in the main structure of the compound to industrially important entity by means of plant enzymes [3, 50].

The use of biotransformation techniques in the synthesis and modification of pharmaceutical drugs is considered as one branch of green chemistry. Biotransformation eliminates the need for the use of

toxic metal catalysts, flammable solvents, and harsh conditions (e.g. high pressure, high and low temperatures) [4, 51]. The use of plant cell culture for biotransformation requires the selection of cell types that express the enzymatic capabilities to catalyze the specific reaction of interest. Another factor in the cell selection process is the specificity of the enzyme reaction. Plant cells have been shown to perform more than one transformation with a given substrate. Therefore, the selection process needs to focus on the detection of cell lines that specifically catalyze the desired reaction with little or no contamination from other reaction products [5, 52, 53, 54, 55, 56, 57, 58, 59, 50, 61, 6].

There are two main reasons to choose plant cells for biotransformation processes: Firstly, these cells are generally able to catalyze the reactions stereo-specifically, resulting in pure products. Secondly, they can perform region-specific modifications that are not easily carried out by chemical synthesis or by microorganisms. These reactions include reduction, oxidation, hydroxylation, acetylation, esterification, glycosylation, isomerization, methylation, demethylation, epoxidation, etc. The presence of biotransformation potential in plant cells is a necessary condition for practical application. Advantages of biotransformation include enhancement in the productivity of the desired compound and the production of novel compounds.

For a successful process, the following prerequisites must be met: the culture must have the necessary enzymes; the substrate or the precursor must not be toxic to the culture; the substrate must reach the cellular compartment of the cell and the rate of product formation must be faster than its further metabolism. Biotransformation utilizing microorganisms bacteria and fungi is a routine process for the synthesis and modification of many drugs and natural products [7].

1.1. METABOLISM AND BIOTRANSFORMATION OF PHARMACEUTICAL DRUGS

Drugs and natural products extracted from plants undergo biotransformation inside the body by natural enzymes. This biotransformation process aims to defend the body against invading xenobiotics, improve the hydrophilicity of xenobiotics, and facilitate their excretion. Because of this process, the drugs are converted into metabolically more or less active entities, detoxified and/or changed their pharmacokinetic properties. To catalyze these reactions, the body utilizes a huge set of enzyme systems. In almost all cases, the biotransformation products are more water soluble than parent molecules and thus can be handled more easily by excretory organs like the kidney. Total xenobiotics load can also be minimized by the transformation of xenobiotic substances to multiple products [8]. Biotransformation or metabolism is the major clearance mechanism of most drugs. Biotransformation reactions of pharmaceutical drugs can be accompanied by various events as:

A- Formation of more stable metabolites which are devoid of pharmacological or toxicological activity.

B- Formation of short living chemically reactive metabolites which can have a toxic effect.

C- Formation of chemically stable metabolites with unpredictable pharmacological activity.

The formed metabolites can significantly alter the targeted pharmacological action. These metabolites may possess more potent agonist action leading to enhanced pharmacological effect or it may lead to the total inhibition of the pharmacological activity of the administered drug. It is estimated that 22% of the top 50 drugs in the United States of America undergoes biotransformation reactions inside the body leading to the formation of active metabolites altering the overall pharmacological effect of these drugs. The most important group of enzymes responsible for metabolizing xenobiotics is cytochrome P_{450} [9].

Plant tissue culture has many advantages over traditional methods of propagation and these advantages can be summarized in the following points:

A- The production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits.

B- Quickly produce mature plants.

C- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.

D- The regeneration of the whole plant from plant cells that have been genetically modified.

E- The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests and pathogens.

F- The production of plants from seeds that otherwise have very low chances of growing

G- To clean plant of viral and other infections and to quickly multiply these plants as cleaned stock for horticulture and agriculture [6, 62, 63, 64].

Several plant tissue cultures rich in enzymes will be tested for the modification of certain pharmaceutical drugs. Plants cell cultures as *Catharanthus roseus*, *Daucus carota*, *Nicotiana tabacum*, etc, are selected based on their content of interesting enzymes for biotransformation process [7, 8, 9, 53, 54, 65, 66]. The enzymes in the selected plants are well defined and tested previously in several manuscripts for the modification of different pharmaceutical drugs and

natural products.

2. TAXOL

2.1. Taxol a Powerful Anticancer Drug

Cancer can be defined as a group of diseases and pathological conditions which are characterized by uncontrolled growth and spread of living cells. This uncontrolled spread may lead to death if not controlled or stopped. Causes of cancer can be external (tobacco smoke, pathological microorganisms, toxic chemicals, and harmful radiation) or internal (mutant genes, unbalanced hormones, and autoimmune diseases). Cancer can be treated utilizing surgery, radiation, and cytotoxic drugs. The National Institute of Health estimated that the total cost of cancer is $228.1 billion: $93.2 billion for direct medical costs; $18.8 billion for indirect morbidity costs for the year 2008 [10].

The serious effects of cancer on human beings and society were the driving forces for scientists to devote their efforts to discover new therapeutic agents to treat this disastrous disease. Brest cancer represents one of the major causes of death among women in Egypt. It represents (32.7%) of the cancer cases among Egyptian women, followed by central nervous system cancers (7.2%), non-Hodgkin lymphomas (6.3%), cervix cancer (4.5%) and cancers of the colon and rectum (4.2%) [11]. The fight against cancer with chemotherapeutic drugs is frequently hindered by either intrinsic or acquired resistance of the tumor cells. In both cases, the tumor can become resistant to a variety of antineoplastic drugs of varying structures and mechanisms of action, a process termed multi-drug resistance (MDR). Although this phenomenon can develop by several different mechanisms, a common cause is believed to be overexpression of certain plasma membrane glycoprotein (P-gp), a transporter protein that acts as an energy-dependent drug efflux pump, hindering the adequate intracellular accumulation of a broad range of cytotoxic drugs. Pharmaceutical companies and research laboratories are in a continuous battle to find new drug entities to treat different types of cancer and find derivatives to fight against MDR developed cancer [12].

One of the milestones in the war against cancer was the discovery of Taxol (Figure 1). Taxol (paclitaxel), a taxane diterpenoid, is one of the most potent anticancer drugs discovered in the twentieth century. It shows cytotoxic activity against leukemia cells and inhibitory action against a variety of tumors such as ovarian cancer and has been recognized as one of the most effective and widely used anticancer agents [13, 67, 68, 69, 70]. This drug is originally isolated from the bark of western yew (Pacific yew) *Taxus brevifolia* which is the only FDA-approved source for clinical use. Taxol (generic name paclitaxel) is one of the structurally more complex representatives of the approximately 400 defined taxoids (i.e., taxane diterpenoids) produced by Taxus (yew) species all of which are based on the unusual taxane (pentamethyl[9.3.1.0]3,8tricyclopentadecane) skeleton, or rearrangement products of this tricyclic scaffold [14]. Taxol binds to the tubulin polymer and inhibits its depolymerization. In vitro, it enhances the polymerization of tubulin, and those microtubules formed in the presence of the drug possess unusual stability and resist depolymerization by cold temperature, dilution, and Ca^{2+} (they are organized in extremely stable bundles). The drug is a potent inhibitor of eukaryotic cell division causing a block in the late G2/M phase, however, after the disruption of microtubules, the precise means by which cell death occurs are not clear. In HeLa cells, low concentration of Taxol induces a cell-cycle block at the metaphase/anaphase transition of mitosis [15].

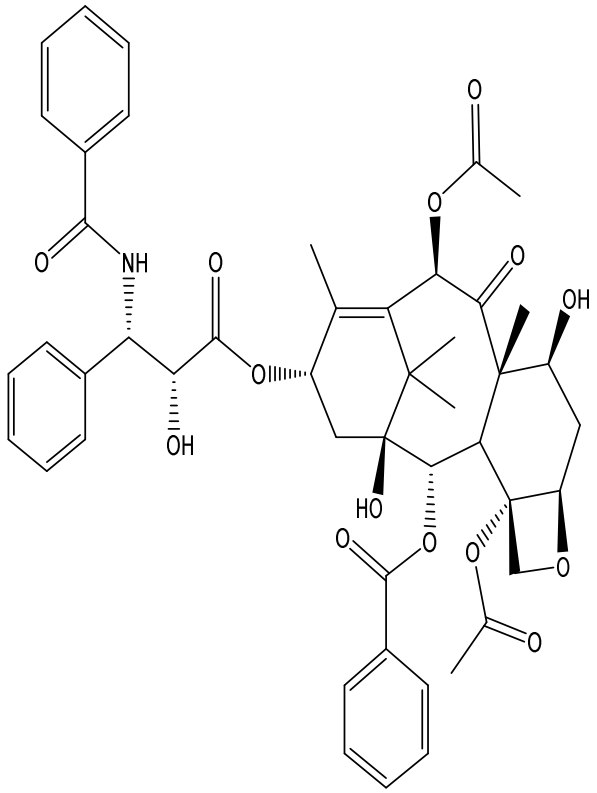

Figure 1: Taxol

2.2. Taxol Side Effects

Taxol is hydrophobic in nature and shows limited water solubility. To improve Taxol solubility, it is formulated with polyethoxylated castor oil (Cremphor EL) containing 50% ethanol. It seems that the serious hypersensitivity of Taxol may be related to the Camphor EL vehicle. Aiming to alleviate serious side

effects of hypersensitivity of Taxol additives, it is administered after premedication with antihistamines, corticosteroids, etc. Other drawbacks associated with Taxol are:

1. Debilitating side effects (aching or painting in joints and muscles, skin changes, numbness or tingling in hands and feet, headaches, allergic reactions, nausea, and vomiting).
2. Taxol is powerless against some cancers, e.g., colon cancer.
3. Structural modifications of the parent structure are not trivial due to the high complexity of the structure and the presence of many stereogenic centers.
4. Therapeutic failure with the use of Taxol (failure to kill all cancer cells), can lead to disastrous side effects as the development of resistant mutant cells or metastasis of the cancer cells to other organs.

2.3. Structural Activity Relationship of Taxol

Taxol was subjected to intensive investigation to determine the active sites in the structure which are behind the cytotoxic effect of this drug [16, 71, 72]. Some derivatives of Taxol show no significant loss of activity (for example, 7-OH can be esterified, epimerized or even removed). Studies have shown that the side-chain at C13 has one of the requirements for biological activity (Baccatain III is not cytotoxic and is totally inactive in vitro toward microtubule assembly). The C-2 benzoyl group and an intact oxetane ring are essential for cytotoxicity and stabilization of microtubules. Substituents on the C-2 benzoyl group have profound effects on the biological activity of Taxol. In vitro studies showed that Taxol is extensively metabolized in humans by liver cytochrome P_{450} enzymes [17].

2.4. Taxol Solubility

Several research groups have investigated the possibility of increasing Taxol water solubility while maintaining its cytotoxicity through several methods. Hayashi et al. designed and synthesized of isotaxel as a novel approach of water-soluble paclitaxel prodrug with no auxiliary and no byproduct. Taxol shows sparing water solubility 0.00025 mg/ml. Isotaxel is 2'-O-benzoyl isoform of Taxol with ionized 3' amino group that converts to Taxol by O-N acyl migration (pH-dependent). No additional water-solubilizing auxiliaries, no byproducts and no added detergents (no side effects) [18]. Hamada et al. enhanced water-solubility and bioactivity of paclitaxel using modified cyclodextrin to form paclitaxel-CD inclusion complex of improved solubility. The structural activity relationship is still under investigation [19]. Shimoda et al. made a chemo-enzymatic synthesis of ester-linked Taxol oligosaccharide conjugates as potential prodrugs. One conjugate has water solubility 53 folds higher than that of paclitaxel. C-7 modification of paclitaxel with a longer oligosaccharide chain decreased its cytotoxicity. The oligosaccharide-based transporters have been reported to serve as a target drug delivery system to a specific organ in the living body, liver, in which oligosaccharide moiety can be cleaved by hydrolytic enzymes [20].

2.5. Taxol Biotransformation

Following the determination of the structural activity relationship of Taxol, several research groups studied the effect of modifying the structure of Taxol either synthetically or utilizing biotransformation techniques. Bioconversion of Taxol/cephalomannine by Streptomyces sp. MA 7065 resulted in hydroxylation on the 10-acetyl methyl group in 60% yield and on the benzene ring at the para position of the phenylisoserine side chain in 10% yield. This culture could also hydroxylate the allylic methyl group of the phenylisoserine side chain of cephalomannine quantitatively. All three metabolites were cytotoxic toward human lung, breast, and colon tumor cell lines [21].

Biotransformation of paclitaxel by cell suspension cultures of *Marchantia polymorpha* was studied and it was found that liverwort cultures convert paclitaxel to 7-epi-paclitaxel in good yield and regioselectively hydrolyzes the ester group at the 10-position of paclitaxel [22]. The biotransformation of paclitaxel (Taxol) by the cell suspension cultures of *Rauwolfia serpentina* was also investigated. Three Paclitaxel-based intracellular metabolites were detected from the cell filter cake and were, by high field 1H-NMR and MS data, identified as 10-deacetyltaxol, baccatin and 10-deacetylbaccatin [23]. Hamada et al. studied the biotransformation of Taxol by *Eucalyptus perriniana* cell suspension culture, Taxol was converted to baccatin III, 10-deacetylbaccatin III and 2-debenzoyltaxol [24]. Dai et al. tested the use of *Catharanthus roseus* cell suspension cultures for the biotransformation of Taxol derivatives. The use of this plant cell culture resulted in the formation of four metabolites. The rate of formation of these metabolites was investigated and it was revealed that the administration of the starting material to the cell culture at different stages resulted in changing the rate of formation of these metabolites [25]. In 2008, they reported the biotransformation of four Taxol derivatives utilizing microbial/plant whole-cell enzymatic transformation. They reported the formation of 53 derivatives, 41 of these metabolites were new. The reactions which led to the formation of these metabolites were divergence, e.g. hydroxylation, epoxidation, oxidation, hydrolysis, acylation, O-alkylation, O-glycosylation, rearrangement [26].

3. ATORVASTATIN

3.1. Hyperlipidemia

Hyperlipidemia is the elevation of plasma cholesterol, triglycerides (TGs), or both, or a low high-density lipoprotein level that contributes to the development of atherosclerosis. Causes may be primary (genetic) or secondary. Diagnosis is done by measuring plasma levels of total cholesterol, TGs, and individual lipoproteins. Treatment is primarily based on dietary changes, exercise, and lipid-lowering drugs. There is no natural cutoff between normal and abnormal lipid levels because lipid measurements are continuous. A linear relation probably exists between lipid levels and cardiovascular risk, so many people with "normal" cholesterol levels benefit from achieving still lower levels. Consequently, there are no numeric definitions of dyslipidemia; the term is applied to lipid levels for which treatment has proven beneficial. Proof of benefit is strongest for lowering elevated low-density lipoprotein (LDL) levels. In the overall population, the evidence is less strong for a benefit from lowering elevated TG and increasing low high-density lipoprotein (HDL) levels, in part because elevated TG and low HDL levels are more predictive of cardiovascular risk in women than in men [27].

3.2. HMGCo-A Reductase Inhibitors

HMGCo-A Reductase enzyme is the rate-limiting enzyme in the cholesterol biosynthesis as it is responsible for the conversion of HMG-CoA to mevalonate. Inhibitors of 3-hydroxy 3-methylglutaryl coenzyme A (HMG-CoA) reductase are the primary line of defense for the treatment of hypercholestermia. These inhibitors are known as Statins, which performs the following main functions:

1. Statins lower the level of LDL-C: The structural similarity of 3-hydroxy-3-methylglutaric acid with Statins leads to a competitive inhibition to HMG-CoA reductase enzyme which is the rate-limiting enzyme of the mevalonate pathway thus suppressing new cholesterol biosynthesis.
2. Statins show anti-inflammatory effect: They act as anti-inflammatory and anti-thrombotic which are critical symptoms in patients suffering from hyperlipidemia.
3. Statins improve endothelial function (the main cause of atherosclerosis): Statins stimulate the endothelial nitric oxide synthetase (ENOS) and block the reactive oxygen species production which improves the endothelial lining structure [28, 73].

3.3. Synthesis of Atorvastatin

Moreover, Atorvastatin (see Figure 2) has (3R,5S)-dihydroxyhexanoate side chain, which is the key step in its synthesis as it contains two stereogenic centers. It is either synthesized through racemic resolution [29] or through asymmetric synthesis[30] Both routes have several drawbacks of using toxic reagents and drastic reaction conditions. Recently the biocatalytic approach was utilized for its synthesis which has several advantages over classical methods of synthesis as biocatalysts have high regio- and stereo-selective nature, fewer byproducts are produced, and enzymatic reactions are usually carried at ambient temperature thus reducing the overall energy consumption [31].

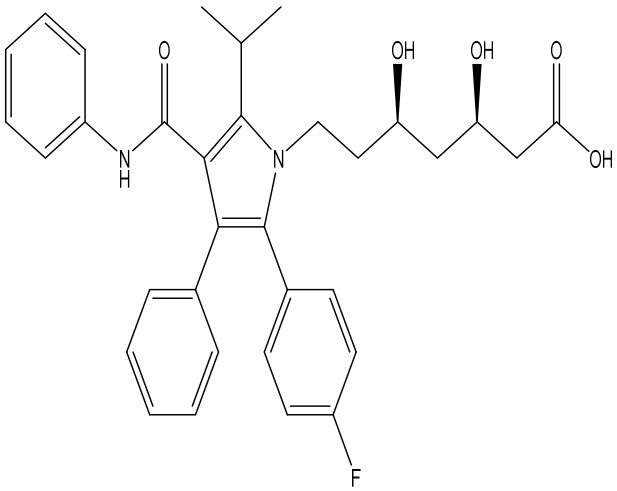

Figure 2: Atorvastatin

3.4. Metabolism and Elimination of Atorvastatin

3.4.1. Metabolism

Atorvastatin undergoes oxidative metabolism by hydroxylation with cytochrome P_{450} as the main route of metabolism. The hydroxylated metabolite is the active metabolite which acts as a cholesterol-lowering drug. It is metabolized to ortho and para-hydroxylated derivative according to the following abundance:

Ortho-hydroxyl derivative > para- hydroxyl derivative > ortho-hydroxyl glucuronate > unchanged > β-oxidation

3.4.1. Elimination

The man route of elimination is through biliary excretion which follows hepatic and/or extra-hepatic metabolism. It is found that bile route of drug-derived excretion, accounting for 73 and 33% of the oral dose in the rat and dog, respectively. Less than 2% of the drug is found in urine [32]. To the best of our knowledge, there are no previous studies were published on the modification of Atorvastatin using plant cell tissue culture.

4. LORATADINE AND DESLORATADINE

4.1. Allergy

Allergy is a word which is usually used to describe a group of unpleasant or dangerous symptoms which a few people get from substances which are harmless to others. Allergy can be defined as any kind of altered state of the immune system in which it reacts differently to a substance as a result of the previous contact. To refer to illnesses brought on in this way, the expression "allergic disease" is commonly used. There is evidence that allergic diseases are increasing all over the world. Allergy seems to be a problem of modern societies. The most affected segment of society is children. Allergy is characterized by a group of unpleasant symptoms as rash, congestion, swelling, flare, itchy and watery eyes.

A fascinating new study by the Asthma and Allergy Foundation of America found that most patients are interested in finding a new prescription allergy medication, mainly because they are not happy with the prescription allergy medication they are taking now. In the 2005 survey, 31 percent of respondents said they are not fully satisfied with their current prescription allergy medication, and 60 percent said they are very interested in finding new allergy medications. Additional results from the survey of 1,214 allergy patients found that 55% believe their current prescription allergy medication does not alleviate their symptoms for a long enough period. Another 44% said their prescription allergy medication does not work fast enough, and 42% are confused by the different prescription allergy medication options available on the market today. This finding motivates pharmaceutical companies to discover new therapeutic entities for alleviating the unpleasant symptoms of allergy.

4.2. Antihistaminic Drugs

Histamine is a chemical messenger that communicates information from one cell to the other. It is a beta-imidazolyl ethylamine derivative which is obtained from histidine amino acid by decarboxylation. It is stored in an inactive bound form and is released as a result of Antigen-Antibody reaction due to different stimuli (external or internal) such as venom, toxins, proteolytic enzymes, detergents, food, and numerous chemicals. Antigen-Antibody reaction leads to the alteration of the mast cell membrane, therefore, histamine will be released causing the unwanted signs and symptoms of allergy. Anti-allergic drugs can be classified into two main categories; mast cell stabilizers and H1 antagonists which Loratadine and Desloratadine belong to this group. The first generation of H1 antagonists was developed in the thirties of the last century by Daniel Bovet. The major side effect of the first generation antihistaminic was the sedative effect due to the effect on the cerebral H1 receptors as they cross the blood-brain barrier (BBB) [33, 34, 74, 75].

4.2.1. Loratadine: Claritine®

Loratadine (see Figure 3) is the drug of choice to relieve sneezing, itching, and rhinorrhea but not as nasal decongestant as leukotriene modifier or glucocorticoids. On increasing the dose administered, it is considered the drug of choice to treat the seasonal and chronic allergic rhinitis and no need for β2 agonists. It is also the drug of choice to treat erythema, wheal and flare symptoms by reducing the number, size, and duration of chronic and acute urticaria. It is administered orally in conjunction with topical glucocorticoids to relief the itching in atopic dermatitis. It has less potential to cross the BBB even at high doses due to its physicochemical character (high plasma protein binding and moderate lipophilicity), thus does not cause the side effects of the 1st generation which is sedation and drowsiness [35, 76].

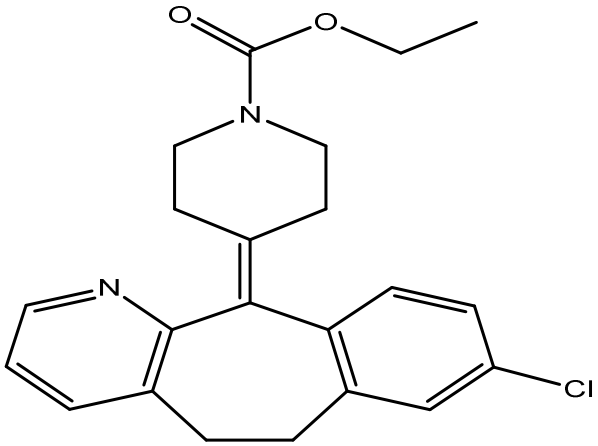

Figure 3: Loratadine

4.2.2. Desloratadine: Aerius® /Deslarex®/Desa®

It is 20 times more active than Loratadine. It is obtained by hydrolysis from Loratadine. It is sympathomimetic so it has a decongestant action. It does not have any adverse cardiovascular effects or cause the heart conduction changes that have occurred with other second-generation antihistamines. Loratadine and Desloratadine (Figure 4) are considered safe drugs and have been transformed from prescription-only status to over-the-counter (OTC) status [36].

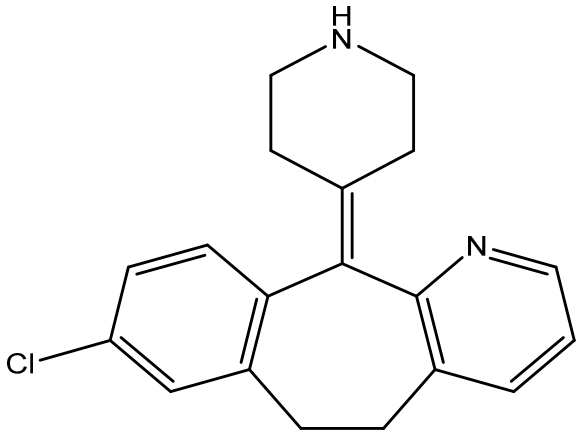

Figure 4: Desloratadine

4.3. Metabolism and Elimination

4.3.1. Metabolism

Desloratadine is the hydrolyzed product of Loratadine. The main enzyme responsible for the Loratadine and Desloratadine metabolism is cytochrome P450-3A4 which metabolizes these drugs by oxidation, even when the drug concentration diminishes its effect is still long lasting depending on the concentration at steady state [37].

4.3.2. Elimination

Loratadine and Desloratadine are both considered the drugs of choice for allergic conditions as they both have the longest elimination half-lives (11 for the former and 26.8 for the later) and rarely eliminated unchanged so the human body gets complete benefit from the administered drug. They are administered once daily so achieve patient compliance and their effect last for a whole day with the least possible drug interactions even if the normal metabolic pathway is interrupted (CYP 3A4) the body can shift to another cytochrome (CYP 2D6) [38].

4.4. Desloratadine Synthesis

Desloratadine is synthesized chemically through multi-step reaction. It can be prepared by Tris-triphenylphosphine ruthenium (II) chloride catalyzed exchange with tritiated water [39]. It can be synthesized also through hydrolysis of Loratadine with alkali potassium hydroxide in 80% ethanol to give Desloratadine (2) in 85% yield, which can be refined by recrystallization in ethyl acetate [40].

5. CONCLUSIONS

The conversion of a chemical molecule by means of biological system is interesting field which holds strong potential. Living plant cells is considered as a bio-synthetic laboratory not only for the primary metabolites, secondary metabolites but also for xenobiotics like pharmaceutical drugs. In biotransformation more than one reaction can be accomplished using cell cultures that express a series of enzyme activities. Plant cell cultures can be used to synthesize the desired product using appropriate precursor.

The process of biotransformation may be simple and scalable where the process is mediated by one or more enzymes with many steps. Single step biotransformation is highly efficient and desirable however, the yield decreases with more steps. Until now, this branch of science did not receive proper attention despite its powerful applications. Taxol, atorvastatin, loratadine, desloratadine are examples of pharmaceutically important drugs which possess potent activity and certain side effects. The use of plant tissue culture may provide a good solution for the synthesis and modification of these important drugs. This arena is still untapped and needs extensive exploration to reveal its full potential.

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