MICROBIAL BIOTRANSFORMATION:
A TOOL FOR DRUG DESIGNING (REVIEW)

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For centuries microbial biotransformation has proved to be an imperative tool in alleviating the production of various chemicals used in food, pharmaceutical, agrochemical and other industries. In the field of pharmaceutical research and development, biotransformation studies have been extensively applied to investigate the metabolism of compounds (leads, lead candidates, etc.) using animal models. The microbial biotransformation phenomenon is then commonly employed in comparing metabolic pathways of drugs and scaling up the metabolites of interest discovered in these animal models for further pharmacological and toxicological evaluation. Microorganisms can conveniently afford drugs difficult obtained via synthesis. The plethora of reported microbial biotransformations along with its added benefits has already invoked further research in bioconversion of novel and structurally complex drugs. This review alternatively discusses the prospect of microbial biotransformation studies as a significant element ameliorating drug discovery and design in terms of cost-effectiveness, environment protection and greater structural diversity as compared to animal models used to study metabolism. To explicate the microbial biotransformation paradigm in drug designing 3 main areas in this aspect have been analyzed: 1 — lead expansion: obtaining pharmacologically improved metabolites from bioactive molecules; 2 — biosynthesis of precursors/intermediates involved in the production of bioactive molecules; 3 — resolution of racemic mixture to obtain enantiomers possessing different pharmacological profiles.

Biotransformations are chemical reactions catalyzed by microbial cells (growing or resting) or enzymes isolated from microorganisms. Drug biotransformation is generally considered to detoxify the drug to form more polar metabolites which can be easily excreted. However, it can also lead to the formation of metabolites possessing greater pharmacological activity than the parent compound or, alternatively, it may prove to be more toxic [1]. Active metabolites may possess on-target activity (significant or entire contribution in pharmacological action) or off-target activity (unrelated to the activity of the parent drug). In some cases metabolites formed might reverse the action of the parent drug [2].

Animal models (hepatocytes, subcellular fractions, liver slices) have been used extensively for studying drug metabolism but microorganisms could be used for the production of mammalian metabolites too. Cytochrome P450-dependent enzymes have been discovered in a variety of yeast, bacteria and fungi possessing the capability to mimic mammalian metabolic reactions partially or completely. Mammalian biotransformation is generally categorized in the phase I and phase II reactions [1].

Phase I reactions. This type of the reactions is called as functionalization since they introduce a functional group in the molecule resulting in a slight increase in hydrophillicity and may increase its pharmacological activity. These are further classified as:

Hydrolysis.

— Carboxylesterases, cholinesterases, organophosphotases, e.g. hydrolysis of procaine (used as local anaesthetic);

— Peptidases;

— Epoxide hydrolases:

— detoxifying enzyme for epoxides (aromatic, unstable and reactive molecules);

— formation of diols (accessible to phase II).

Reduction (azo- and nitro-reductions)

— Enzymes of intestinal flora (especially in large intestine);

— Cytochrome P450 (usually oxidizing enzyme), has the capacity to reduce xenobiotics under low oxygen or anaerobic conditions;

— Interactions with reducing agents (reduced forms of glutathione, NADP).

Oxidations.

These reactions include hydroxylation, epoxidation, oxidation of alcohols and aldehydes, oxidative degradation of alkyl chains, oxidative deamination.

Phase-II reactions. This type of the reactions generally further increases the hydrophillicity of the drug and facilitates the excretion of the drug and its metab-

olites. They are classified on the basis of conjugation of drug molecule or phase−I metabolite with endogenous substances and include the glucuronide, sulfate, glutathione and amino acid conjugations.

Biotransformation is crucial for estimation of specific clinical parameters of the drugs. High bioavailability and clearance usually results from high metabolism, thus establishing the fact that metabolite studies are an important factor in drug designing [1]. Identification of active metabolite is necessary when a drug exhibits unexpectedly enhanced pharmacological activity *in vivo* [2]. Initially, the purpose of microbial biotransformation was to obtain more active or less toxic metabolites. Metabolites obtained through microbial transformation could help to correlate with those obtained through *in vivo* or *in vitro* animal models. When drug metabolism is studied, microbial biotransformation offers several advantages as compared to mammalian metabolism:

1. Simple and cheap maintenance of microbial cultures as compared to cell or tissue cultures or laboratory animals.

2. Facile repetitive screening process in which different strains are used to metabolize the drug.

3. Novel metabolites showing off-target pharmacology.

4. Novel metabolites superceding the pharmacological activity of its parent molecule.

5. Less toxic novel metabolites as compared to parent molecule.

6. Mild and ecologically harmless reaction conditions (normal pressure, low temperature, neutral pH) for sustainability.

7. Dependence on the nature of the biocatalyst and substrate prediction of the metabolic reactions.

8. Convenient scaling up of the metabolite production for pharmacological and toxicological evaluation, isolation and structure elucidation when parallel animal metabolic studies reveal the required information about the metabolites.

9. Generation of structural diversity in a chemical library through introduction of functional groups at various positions of a drug molecule thus in turn affecting the structure-activity relationships.

10. Suitable alternative where it is tedious to introduce a functional group by chemical methods, e.g. $11-\alpha$ and or $11-\beta$-hydroxylation of corticosteroids. It also liberates from use of hazardous chemicals and catalysts thus provide a relatively more safe and efficient method.

11. High stereospecificity of reaction due to the complex, three dimensional and asymmetric nature of enzyme enabling to recognize its substrate and even distinguish different stereochemical configurations of the substrate molecule [3].

12. High regiospecificity as an enzyme specifically attacks its substrate at the position where the reaction takes place [3].

13. Mostly mild incubation conditions.

Biotransformation undoubtedly is a phenomenon that engulfs the solutions to major economic and financial problems faced by pharmaceutical industries regarding the discovery and synthesis of new molecules having the desired characteristics to be launched as an active drug in the market. Although biotransformation encompasses various fields and objectives, the focus of this article aims at 3 main objectives: (1) lead expansion: obtaining more active or less toxic metabolites from bioactive molecules; (2) biosynthesis of precursors/intermediates involved in the production of bioactive molecules; (3) stereochemical reactions and resolution of racemic mixture.

LEAD EXPANSION: OBTAINING PHARMACOLOGICALLY IMPROVED METABOLITES FROM BIOACTIVE MOLECULES

Biotransformations enhance the molecular diversity around active core structures after initial screening or after selecting compounds for preclinical development. In the initial lead expansion phase, the biotransformations can be utilized as a tool for drug designing, leading to substitutions at positions difficult to access by synthetic approaches. These derivatives help refine the structure-activity relationships, potentially generating new ideas of compounds to be synthesized. Also, the biotransformations are propitiously combined with synthesis, as in most cases reactions can be applied to related structures, thus multiplying the number of available compounds for screening.

Metabolites exhibiting significant pharmacological activity or less toxicity as compared to parent molecule could be expediently used as leads for drug designing. Structural modification during lead optimization phase of drug discovery might improve desired properties of lead candidates. Accordingly, if a metabolite with ample pharmacological activity and less toxicity is discovered, it might serve as a lead with an additional benefit of advanced properties. Such approach was used in the discovery of ezetimibe, a cholesterol absorption inhibitor [2]. In this study an active metabolite (lead candidate) which was 30 times more potent than the parent molecule was further optimized to produce final drug candidate which was 400 times more potent than the original lead.

Metabolites apart from possessing specific inherent distinction in its chemical characteristics from the parent drug also acquire structural similarity to the parent molecule. Hence, these metabolites show certain pharmacological characteristics similar to the parent molecule. This is mostly observed in simple functionalization reactions, e.g. O-demethylation, N-demethylation,

hydroxylation and dehydrogenation. A minor structural modification of the metabolite may cause loss of potency or modification of pharmacological activity of the parent drug. For example, O-demethylation of venlafaxine leads to an active metabolite but N-demethylation results in loss of activity [2].

Several factors need to be considered during designing drugs via microbial biotransformation. For instance, if functionalization reaction happens at the auxophoric (non-pharmacophoric) group that does not obstruct binding of the parent molecule to the receptor or enzyme, or it leads to optimization of metabolite binding, that could be expected to retain or enhance the activity of the parent compound. On the contrary, a decline in potency is to be anticipated if a functionalization reaction results in the development of auxophoric group that hinders with binding of the drug to the target or a pharmacophoric group undergoes biotransformation.

Metabolites may acquire extensive array of pharmacological activities depending on structural resemblance to the parent molecule and conservation or optimization of bioactive conformation of the parent molecule [2].

For further clarification and comprehension of the concept of biotransformation regarding its pertinence for attaining metabolites of interest from known pharmacoactive compounds or those undergoing clinical trials study of lead expansion phase of drug designing is further divided into 2 categories with regard to the origin of molecule as a natural and synthetic drugs.

Natural drugs. For thousands of years, natural products have played a significant role all over the world in treating and preventing human diseases. Natural product medicines have come from diverse source materials including terrestrial plants, vertebrates, invertebrates and microorganisms and marine organisms [4]. These are additionally classified according to their chemical nature as alkaloids, glycosides, flavonoids and terpenoids.

Alkaloids are a collection of complex nitrogen-containing organic compounds derived from a variety of sources, including microorganisms, marine organisms and plants, via complex biosynthetic pathways. They find a broad range of pharmacological applications in various diseases (malaria, cancer, hypertension) and disorders (Parkinson's disease) [5].

Due to rigid structural conformation of alkaloids, their structural modification was difficult but using the latest molecular techniques such as enzyme expression has eliminated the limits of metabolite designing of alkaloids [5].

El Sayed et al. [6] investigated the transformation of veratramine which is an alkaloid possessing antihypertensive and serotonin agonist activity. Out of 25 species that were screened, *Nocardia* species ATCC 21 145 metabolized veratramine completely into 3

new metabolites which were subsequently tested for antimalarial activity [7].

Orabi et al. [7] have reported the biotransformation of benzosampangine (a), a semisynthetic derivative of sampangine, which possess antimycobacterial activity (Fig. 1). *Cunninghamella blakesleeana* ATCC 8688a was shown to convert the compound to β-glucopyranose conjugate (b).

Papaveraldine, a minor benzylisoquinoline alkaloid isolated from *Papaver somniferum*, was effectively biotransformed to S-papaverinol and S-papaverinol N-oxide by undergoing stereoselective reduction at the ketone group (Fig. 2). Papaveraldine shows antispasmodic effect and protection against histamine-induced bronchospasm. S-papaverinol did not exhibit any significant antimicrobial (against Candida albicans, *Staphyllococcus aureus* and *Pseudomonas aeruginosa*), antiviral (against herpes simplex type 1) or antimalarial (against *Plasmodium falciparium* D6 and W2 clones) activities [8]. These microbial biotransformation results of papaveraldine correlated with the previous plant cell transformation studies on papaverine and isopapaverine [9—12].

Herath et al. [13] studied transformation of Harman alkaloids harmaline, harmalol and harman. Harmaline is a potent monoamine oxidase inhibitor and serotonin antagonist having hallucinogenic activity. *Rhodotorula rubra* was selected out of 37 microbes for preparative scale fermentation of harmaline and harmalol. Harmalol transformed into 2-acetyl-3-(2-acetamidoethyl)-7-hydroxy-indole. Fermentation of harmaline by *R. rubra* gave 2-acetyl-3-(2-acetamidoethyl)-7-methoxyindole which demonstrated antibacterial activity against many Gram positive bacteria and reduced toxicity as compared to using *Cunninghamella echinulata* NRRL 3655. Last microorganism completely converted harman into 2 metabolites, 6-hydroxy-harman and harman N-oxide. These may contribute in the elimination of the parent compound as they are more polar.

Bufadienolides are relatively new steroidal compounds derived from the chinese drug Chan'su. These are C-24 steroids having the distinctive structural feature as a doubly unsaturated 6-membered lactone ring on 17-β-position. They exhibit considerable inhibitory activities against human myeloid leukemia cells and prostate cancer cells [14, 15]. Kamano et al. [16, 17] obtained 80 bufadienolides and studied their structure—activity relationships (SAR) and quantitative structure-activity relationship (QSAR) on the inhibition of colchicines-resistant primary liver carcinoma PLC/PRF/5 cells. It was found that slight changes in functionality of bufadienolides could appreciably modify their cytotoxicities. The critical structural necessities for escalating the inhibitory activities have been recognized. All the test bufadienolides are natural products isolated from Chan'Su, or their chemical derivatives, and the oxyfunctionality sites are restrict-
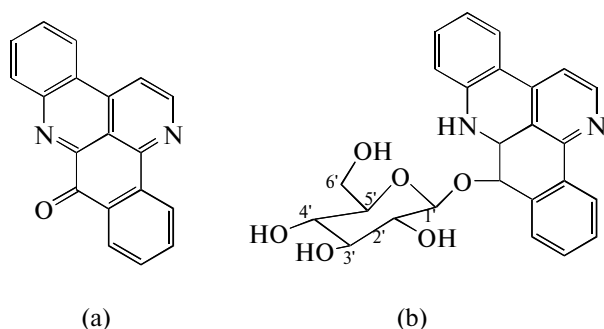
Fig. 1. Structures of benzosampangine (a) and benzosampangine ß-glucopyranoside (b).

	R
Papaveraldine (a)	=O
S-Papaverinol (b)	—OH
S-Papaverinol N-oxide (c)	—OH, N-oxide

Fig. 2. Structures of papaveraldine (a), S-papaverinol (b) and S-papaverinol N-oxide (c).

ed to C-3, C-5, C-15 and C-16-positions. The cytotoxicities of bufadienolides oxygenated at other sites, which are apparently hard to obtain by synthetic techniques remains unknown.

Ye et al. [18] obtained hydroxylated derivatives of bufalin using *Mucor spinosus* as a biocatalyst. The biotransformation products obtained in this study were bufalin derivatives hydroxylated at C-1β, C-7β, C-11β, C-12β, C-15α, C-15β or C-16α positions. All the oxyfunctionalities apart from 5-hydroxylation are novel for natural bufadienolides, and are obviously difcult to obtain by chemical means. It was discovered that hydroxylation of bufalin at different sites could remarkably modify the cytotoxic activities. 1β-hydroxybufalin and 12-β-hydroxy-bufalin showed potent cytotoxicities comparable to bufalin. Both compounds are even more active against the human gastric cancer BGC-823 cells and the human cervical cancer HeLa cells with IC50 values of 8—10 M. Biotransformation of bufalin by *M. spinosus* yielded 12 products including 7 new compounds with novel oxyfunctionalities at 1β-, 7β-, 11β-, 12β-, and 16β-positions. The results of cytotoxicities of 30 bufadienolides *in vitro* revealed that 3-OH glucosylation or hydroxylation at C-1β or C-12β sites might be promising reactions to obtain more polar bufadienolides with enhanced cytotoxic activities.

Zhang et al. [19] investigated biotransformation of 3 cytotoxic bufadienolides, — resibufogenin, cinobufagin and bufalin, by *Nocardia* sp. NRRL 5646. Resibufogenin notably transformed to a metabolite 3-acetyl 15β-hydroxy-bufatolin by means of an unexpected 14β,15β-epoxy ring cleavage and acetoxylation at 16-position. This compound exhibited strong inhibitory activities against the human hepatoma HepG2, human gastric cancer BGC-823 and human cervical carcinoma HeLa cells with IC50 values of 2.8, 0.5 and 3.1 μM, respectively. This increase in cytotoxicity could be attributed to disappearance of 14β,15β-epoxide ring cleavage and presence of 16-acetoxyl and 14β-hydroxyl groups. Cinobufagin and bufalin were
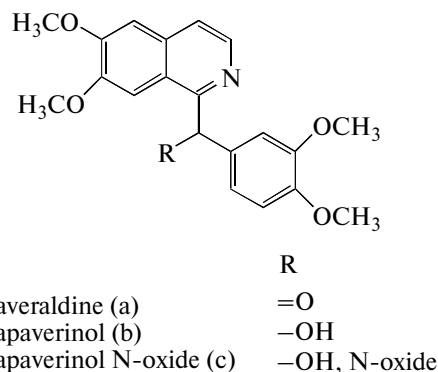
biotransformed in parallel studies resulted in the creation of 3-acetylated metabolites which assayed displayed cytotoxicities weaker than their corresponding parent molecules.

The diterpene ent-pimara-8(14),15-dien-19-oic acid has shown growth inhibition of the cariogenic microorganisms with very satisfying minimal inhibitory concentration values ranging from 2.5 to 5.0 μg/mL [20]. Its metabolism with the aim to produce lead candidates showing better pharmacological characteristics with *Glomerulla cingulata* and *Mucor rouxii* afforded 3 metabolites with one candidate ent-8(14),15-pimaradien-19-ol exhibiting 2-3-fold more activity than its precursor in the time-kill assays [21]. According to Urzúa et al [22], the structural characteristics that endorse the efficient antibacterial activity including significant lipophilicity, capable of insertion into the cell membrane, and one strategically located hydrogen-bond-donor group (HBD; hydrophilic group), which interacts with the phosphorylated groups on the membrane. In this work, it was also emphasized that a second HBD introduced in the lipophilic boundary or the absence of this hydrophilic group in the skeleton led to reduction or inhibition of the activity.

Artemisinin (Fig. 3) has been established as a clinically effective agent for the treatment of chloroquine-resistant malaria, however its low-water solubility has prevented the formulation of an efficient oral dosage form, which consequently has greatly restricted its prevalent use. In addition, it has also proven to be neurotoxic in clinical trials. Hence, it is crucial to have an oral, economical, non-toxic antimalarial drug effective against chloroquine-resistant malaria. As total synthesis of artemisinin does not provide a practical approach for drug design, studies using artemisinin as a natural product scaffold for synthetic manipulation present a logical design approach. Intricacies associated with the synthetic techniques to connect 'synthetic handles' to artemisinin are a limitation to an economically feasible production of any potentially effective artemisinin derivatives. Molecular modeling and SAR ex-
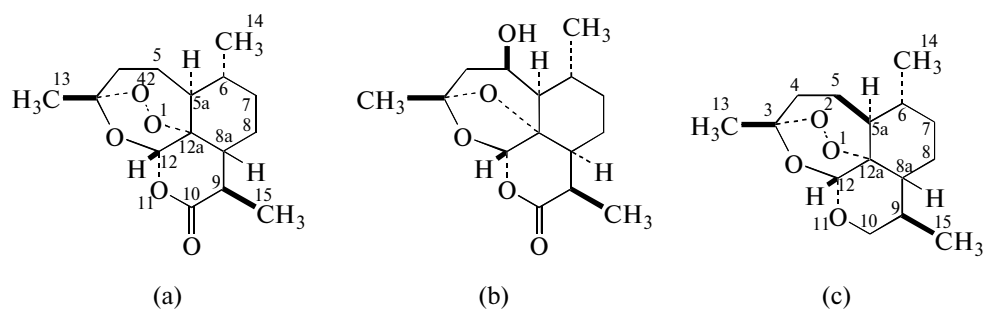
Fig. 3. Structures of artemisinin (a), 5-hydroxyartemisinin (b) and 10-deoxoartemisinin (c).

periments specify that derivatization of artemisinin in positions 4–7 present the greatest potential of enhancing water solubility however preserving its anti-malarial activity. 5-β-hydroxylated derivative of artemisinin has been successfully formed after transformation with *Eurotium amstelodami* and *Aspergillus niger*. Besides this derivative, many other compounds have been produced by SAR guided techniques that exhibit excellent anti-malarial and water solubility profiles [23].

Parshikov et al. [24] have also reported transformation of 10-deoxoartemisinin using *A. niger* into 2 derivatives, 15-hydroxy-10-deoxoartemisinin and 7-β-hydroxy-10-deoxoartemisinin, providing vital scaffolds with the capability of being used for new anti-malarial drugs.

Flavonoids are the most abundant plant derived natural compounds possessing a plethora of unique yet distinct pharmacological uses. Microbes have been extensively used for their derivatization particularly sulfation due to the well-known significance of flavonoid sulfates as potential therapeutic agents [25, 26]. Using *Cunninghamella elegans,* Ibrahim [27, 28] generated sulfated metabolites of naringenin (a potential cytotoxic), chrysin and apigenin, which possess anticarcinogenic, antioxidant, antinflammatory activities besides other activities. Certain regiospecificity of the biocatalyst was noted as all of the substrates were sulfated at C-7 position. Naringenin gave only one metabolite, naringenin-7-sulfate. Fermentation of chrysin gave apigenin-7-sulfate and chrysin-7,4'-disulfate, whereas apigenin transformed to apigenin-7-sulfate and apigenin-7,4'-disulfate. The replacement of chemical synthesis of expensive apigenin by a facile and economical biocatalytic technique was developed [28].

The active form of glycyrrhizin, glycyrrhetinic acid, is known to acquire several pharmacological activities such as anti-ulcerative, anti-inflammatory and immunomodulating effects [29] alongside hepatoprotective and significant antiviral activities, possibly due to immuno-modulating activity through stimulation of nitric oxide production [30]. NO is a host defence molecule produced by the enzyme NO synthase in different im-

mune cells. It has been identied to inhibit the growth of microorganisms including bacteria, fungi and viruses. Maatooq et al. [30] biotransformed glycyrrhetinic acid using 3 microbes which generated 7 metabolites. The major metabolites were 7β,15α-dihydroxy-18β-glycyrrhetinic acid and 1α-hydroxy-18β-glycyrrhetinic acid. Their hepatoprotective activity was assessed via FeCl3/ascorbic acid-induced lipid peroxidation of the normal mice liver homogenate. 7β,15α-dihydroxy-18β-glycyrrhetinic acid showed immense antioxidant activity as compared to its precursor and other major metabolites during CCl4 induced hepatoxicity test. Both of the metabolites along with glycyrrhetinic acid induced NO production remarkably in rat macrophages, thus revealing their potential to be used as efficient hepatoprotective agents.

Hydroxylation and O-methylation of baicalin and baicalein has been observed as an increase of antioxidant activity might be according to hydroxylation in flavones system especially B ring [31]. Baicalin upon fermentation with *Coryneum betulinum* yielded 4',5,6,7-tetrahydroxyflavone whereas *Chaetomium* species yielded 5,7-dihydroxy-6-methoxyflavone. Fermentation of baicalein with *Chaetomium* species and *Cryptosporiopsis radicicola* gave only 5,7-dihydroxy-6-methoxyflavone. *Pennicillium chrysogenum* biotransformed baicalein to 5,7-dihydroxy-4',6-dimethoxyflavone and one metabolite observed with *Chaetomium* species. High regiospecificity was observed for methylation as only C-6 in the A ring underwent methylation [32]. Cannflavin A and B, two methylated isoprenoid flavones, represent the first aglycone flavonoids isolated from *Cannabis sativa*. Efficient antileishmanial activity for cannflavin A and B was reported [33] as IC$_{50}$ 10.3 and 13.6 μM, respectively. Incubation of cannflavin A with *Mucor rammanianus* yielded 6″*S*,7″-dihydroxycannflavin A, 6″*S*,7″-dihydroxycannflavin A 7-sulfate and 6″*S*,7″-dihydroxycannflavin A 4'-*O*-α-L-rhamnopyranoside [34]. *Beauvaria bassiane* transformed cannflavin B to 7-O-β-D-4‴-O-methylglucopyranoside and 7-sulfate. These products were subsequently evaluated for their
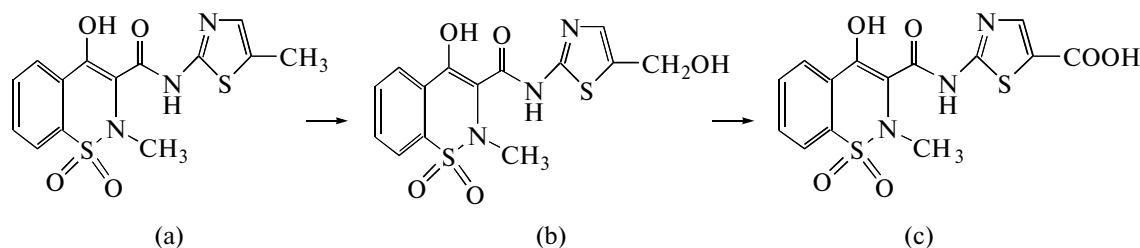
Fig. 4. Structures of meloxicam (a), 5-OH methyl meloxicam (b) and 5-carboxy meloxicam (c).

antifungal, antibacterial antimalarial and antileishmanial activity.

Steroids are the pioneer compounds which proved to be ideal substrates for biocatalysts, thus paving the way for infinite possibilities garnered by the technique of biotransformation. Prednisone was metabolized by *C. elegans* into 2 metabolites: 17,21-dihydroxy-5-pregn-1-ene-3,11,20-trione and 17,20S,21-trihydroxy-5-pregn-1-ene-3,11-dione. Upon incubation with 3 other fungi (*Fusarium lini, Rhizopus stolonifer, Curvularia lunata*) afforded a single metabolite 1,4-pregnadiene-17,20S,21-triol-3,11-dione. This compound proved to be a more potent inhibitor of arachidonate 5-lipoxygenase as compared to prednisone [35].

Mestranol, a well recognized contraceptive, was transformed by *Cunninghamella elegans* into 2 metabolites 6β-hydroxymestranol, a known metabolite, and 6β,12β-dihydroxymestranol, a novel metabolite [36].

7-hydroxy-steroids have been shown to exert neuroprotective effects. In this respect, 5-androstene-3β,7α,17β-triol and 5-androstene-3β,7β,17β-triol have gained popularity. 5-androstene-3β,7β,17β-triol could stimulate concavalin A- and lipopolysaccharide-induced cell proliferation in experiment with cultured murine spleenocytes, and it also countered the effect of cortisol on Con A-activated lymphocyte proliferation as well as glucocorticoid-induced IL-2 and IL-3 production [37—39]. 5-Androstene-3β,17β-diol was synthesized from diosgenin which was then biotransformed to 7-hydroxylated derivatives by *Mucor racemosus* isolated from the soil samples [40].

Recently, Russell et al. [41] have emphasized upon the idea that structural similarity shared by secondary metabolites of the phenyl propanoid pathway consumed in our daily diet with non-steroidal anti-inammatory drugs (NSAIDS) lead to the possibility that they could serve as leads to produce analogues corresponding to NSAIDS in their anti-inflammatory mechanisms. 5-5'-linked dimer of ferulic acid, an abundant secondary metabolite commonly found in diet, was selected as lead to produce metabolites. The metabolites formed displayed significant inhibition of prostanoid production.

Synthetic drugs. Using 2 different strains of *Streptomyces griseus* Gurram et al. [42] have observed that the strain NCIM 2622 biotransformed meloxicam to 5-hydroxymethyl meloxicam (Fig. 4) in a higher yield and 5-carboxy meloxicam in trace amounts. However, the strain NCIM 2623 produced only the first compound in trace amounts. Although oxidative transformations are likely to increase the potency of metabolites, additional pharmacological activities of meloxicam and its metabolites need to be explored. Hence, these studies are in progress [43].

Strong structural resemblance amongst cyclooxygenase (COX)-2 inhibitors has generated the prospective of their potent analogues via biotransformation [44]. Eight metabolites of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide, – celecoxib, were produced after screening with several microorganisms. The hydroxylated and carboxylated derivatives formed had been shown to possess no inhibitory activity for COX-1 and COX-2. Microbial N-acetyltransferase catalysed N4 acetylation at NH_2 position of sulfonamide group of celecoxib and increased COX-1 and COX-2 inhibitory effects 3.3 times.

Ma et al. [45] have reported 7 metabolites of metoprolol, namely O-desmethylmetoprolol, metoprolol acid, α-hydroxymetoprolol, N-desalkylmetoprolol, deaminated metprolol, hydroxyl-O-desmethylmetoprolol and glycosylated O-desmethylmetprolol formed during biotransformation with *C. blakesleeana* provided the opportunity of scaling up the reaction for further pharmacological evaluation and structure elucidation.

Schmitz et al. [46] proposed a viable alternative technique for the synthesis of fexofenadine from terfenadine using a membrane system of *Cunninghamella blakesleeana*. The reaction conditions were optimized and microcrystalline terfenadine was employed to overcome its low water solubility. Although this method failed to produce fexofenadine yet, tertiary butyl alcohol derivative of terfenadine was obtained which upon further reduction and oxidation of reagents could yield fexofenadine. Terfenadine and ebastine are more toxic compared to their active metabolites. Due to arduous chemical synthesis of fexofenadine and carebastine, a facile biocatalytic technique was devised by Mazier et al. [47] in which fermentation media were optimized by the addition of soyabean peptones because of their oxidation inducing capacity. Two bacte-
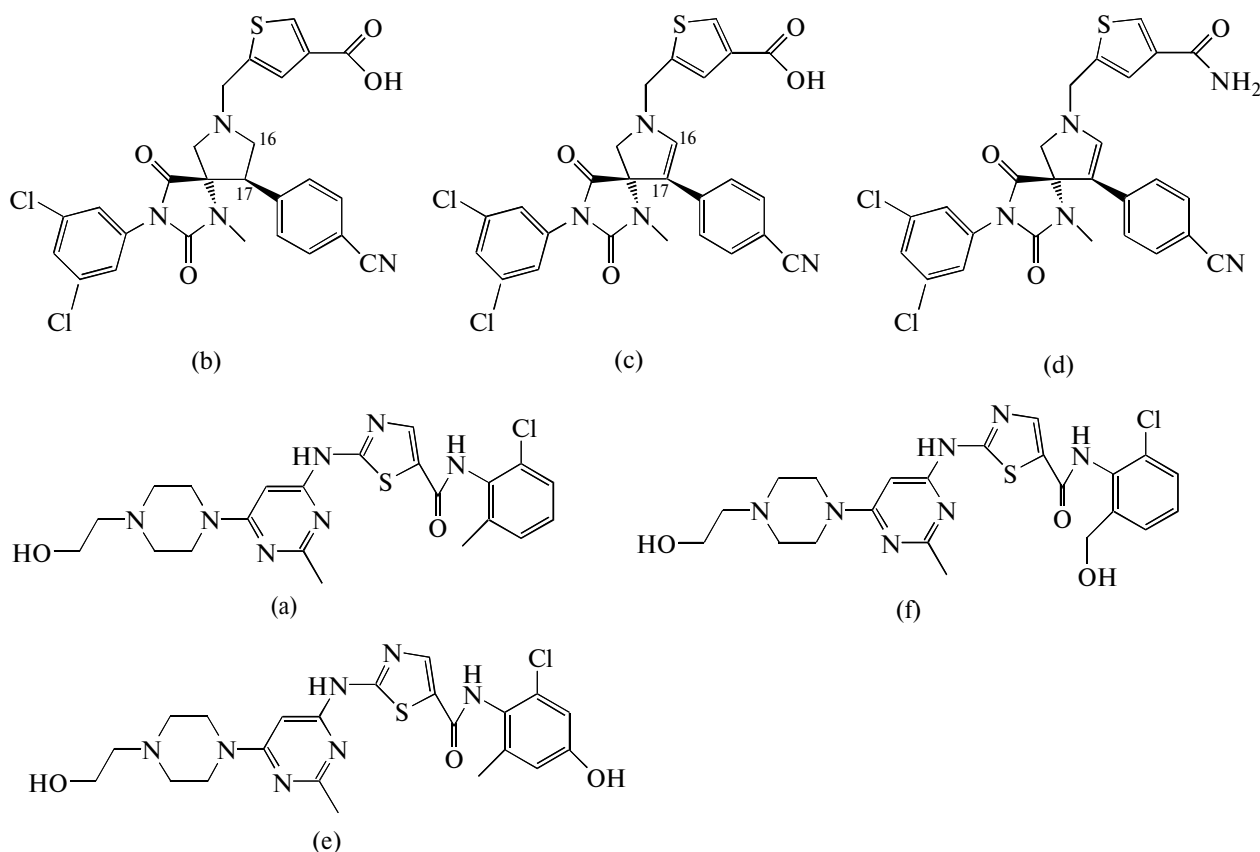
Fig. 5. Structures of dasatinib (a), BMS-587101 (b), M6 (c), amide of M6 (d), M20 (e) and M24 (f).

rial and 3 fungal strains were selected and *Absydia corymbifera* showed the complete transformation of terfenadine and ebastine. Yeast expressing human cytochrome P450 failed to oxidize terfenadine, thus showing its limitation for scaling up the metabolite production. The reason assumed was inability of the substrate to penetrate intact yeast cells [47].

N-acetylation of ciprofloxacin has been reported by *M. rammanianus* thus revealing its possible capability of transforming other flouroquinolones to N-acetylated derivatives [48].

Albendazole, which is a benzimidazole carbamate, possesses broad antiparasitic activity. Its efficiency is due to its main metabolite, albendazole sulfoxide, which also has significant anthelmintic activity. It has greater water solubility profile as compared to the parent molecule. Twelve different bacteria and 5 actinomycetes cultures were screened to produce novel albendazole metabolites. *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* produced albendazole sulfoxide. *Enterobacter aerogenes, Klebsiella aerogenes, Pseudomonas aeruginosa, Streptomyces griseus* transformed albendazole to albendazole sulfoxide and albendazole sulfone [49].

Regioselective oxidation of 3-β-hydroxy-5,16-dien-pregnane-20-one (CDRI 80/574) with *Aspergil-*

lus ochraeus and *A. niger* created 4 metabolites. 11α, 15β-Dihydroxy-4,16-dien-pregnan-3,20-dione was a novel metabolite and 3β,11α-dihydroxy-5,16-dien-pregnane-20-one, a common precursor of many hormonal compounds, was also identified. CDRI 80/574 is a compound of pregnane class which exhibits significant lipid profile lowering via farnesoid X receptor antagonism. It has completed phase-III of clinical trials. Hence, its biotransformation is important with regards to correlation with mammalian metabolism and clinical evaluation of the metabolites [50].

Li et al. [51] have performed rapid synthesis of 3 major human circulating metabolites of drugs undergoing clinical trials, dasatinib and BMS-587101 (Fig. 5), by using *Actinomycetes* genera. A 24-well microtiter plate screening system was developed to screen actinomycete strains efficiently to scrutinize their ability to selectively produce metabolites of interest. Actinomycete strains had 2 benefits over fungi as they avoided possible risk of cross contamination and formation of large mycelium aggregates as observed with fungi.

P450 enzymes, as well as other monooxygenases and dioxygenases, have been identified. On the other hand, to prepare specific key mammalian metabolites, it may be helpful to have an array of microbial strains with different specificities for the production of indi-
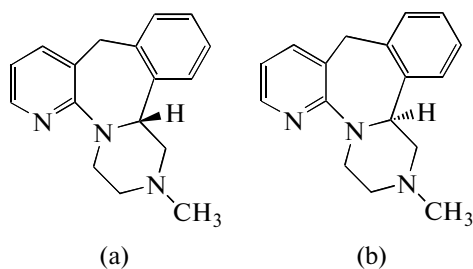
Fig. 6. Structures of R (−)-mirtazapine (a) and S (+)-mirtazapine (b).

vidual metabolites. Selective metabolite formation was demonstrated with diclofenac, the metabolism of which has been well exemplified in diverse biological systems. 24-well microtiter plate system showed that the strains in the wells C2, C5, D1, and D2 selectively prepared 4-hydroxydiclofenac and those in A3, A4 and D5 selectively formed dihydroxydiclofenac but strains in other wells produced non-selectively manifold metabolites. Hence, each actinomycete strain possesses unique oxygenases with different regio- and stereo specificities subsequently corresponding to substrate specificities. So, it is an efficient system ideal for designing the candidate/s of required interest by scaling up the metabolite production with microbes showing enhanced specificity towards the production of desired metabolite/s during screening plate studies. For successful screening plate transformation, it is imperative to consider certain aspects such as selecting strains having the ability to grow in the same medium and possessing the same growth rates. One metabolite of BMS-587101 was determined. It was assumed to be dehydrogenated between C16-C17 after M-S analysis. Enzymes corresponding to human metabolism were identified after transformation of dasatinib as Src and Bcr-Abl-kinases [52].

Metabolites of drugs produced via chemical synthesis or the scaling up of their production from mammalian systems for pharmacological and toxicological evaluation would have been quite expensive. Installation of a double bond between C16-C17 of BMS-587101 through chemical means requires an entirely synthetic route [53]. Therefore, microbial biotransformation method is once again proven to be beneficial in terms of efficiency, safety and cost-effectiveness.

Mirtazapine (Fig. 6) which is commercially available as racemic mixture is clinically used as an antidepressant. Its mechanism of action is attributed to antagonism of α-2 receptors, post-synaptic serotonin type-2 and type-3 receptors, thus reducing undesirable effects [54, 55]. Incubation of racemic mixture of R(−)- and S(+)-mirtazapine with *Cunninghamella elegans* indicated that all the seven metabolites: N-desmethyl-13-hydroxymirtazapine, mirtazapine N-oxide, N-desmethylmirtazapine, 13-hydroxymirtazapine, 12-hydroxymirtazapine, 8-hydroxymirtazapine as

well as the minor metabolite, N-desmethyl-8-hydroxymirtazapine, were formed from the S(+)-enantiomer, with mirtazapine N-oxide as the major metabolite. The R(−) enantiomer formed N-desmethyl-8-hydroxymirtazapine, mirtazapine N-oxide, N-desmethylmirtazapine, and 8-hydroxymirtazapine. 8-Hydroxymirtazapine was the major metabolite [54].

BIOSYNTHESIS OF PRECURSORS/INTERMEDIATES INVOLVED IN THE PRODUCTION OF BIOACTIVE MOLECULES

1-chloro-3-(1-naphthoxy)propan-2-one, a chiral halohydrin, is an important precursor of propranolol. This compound could be obtained by resolution of its racemate by lipases but the major disadvantage is that only 50% optically active product is formed. Whereas whole cell catalyst can yield higher than 50% and high e.e. is achieved [56]. The activity of propranolol resides in S-enantiomer *Saccharomyces cerevisiae* along with other microbes due to differing enantioselectivities were screened to analyze whether they converted halohydrin to R or S propranolol with high optical purity. Two models were designed to study reduction capacity of microbes which consisted of actively fermenting cells and fresh resting cells. Cyclohexanone was employed for preliminary necessary assessment of reducing ability of microbes. The reductive action of each strain was calculated as the percentage of cyclohexanone reduced to cyclohexanol, after 48 h of the addition of the ketone (1 g/L) to the Erlenmeyer culture ask. Using the actively fermenting cells model 1-chloro-3-[1-naphthoxy]propan-2-one was reduced to R-propranolool by *S. cerevisiae* 1317, *Saccharomyces bayanus* 1969, *Psilocybe mexicana* 11015, *Yarrowia lipolytica* 1240. On the other hand, *S. cerevisiae* Type II afforded the S-enantiomer. Production of 2 different enantiomers could be attributed to different enantioselectivities of different enzymes. In fresh resting cells model better results were achieved with cyclohexanone reduction, especially in case of *P. mexicana* 11015 and *Y. lipolytica* 1240. However, the stereoselectivity of the halohydrin precursor reduction in both experimental designs was almost the same. When considering 2 parameters, yield and e.e., resting cells gave better results. *Y. lipolytica* 1240 and *P. mexicana* 11015 gave excellent yields and e.e. which proves that these strains could be used for scaling up the production of S- or R-propranolol from 1-chloro-3-(1-naphthoxy)propan-2-one [57].

Benzohydroxamic acids have an attractive pharmacological profile which comprises of activities such as antimicrobial, antifungal, antifeedant, phytotoxicity and insecticidal [58]. The synthesis of 2,4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one (D-DIBOA) involves 2 steps: (1) nucleophillic substitution of side chain on nitrophenol using ethyl bromoacetate as the reagent and (2) nitro group reduction followed by in-
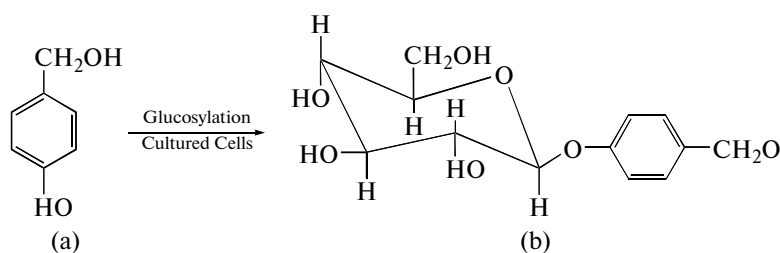
Fig. 7. Structures of *p*-hydroxybenzyl alcohol (a) and gastrodin (b).

tramolecular addition/removal of the ethyl chain from the ester [59]. Vallea et al. [60] have discovered an efficient biocatalytic technique that could replace second step of the chemical synthesis as it employs the use of Pd/C as catalyst and is exothermic in nature. *E. coli* and *Serratia marcescens* were the two strains selected for reduction of the precursor molecule, ethyl 2-(2'-nitrophenoxy)-acetate, as they have previously been used to reduce trinitrotoluene having a structure analogous to D-DIBOA precursor. Esteve-Núñez et al. [61] have demonstrated that nitro group reduction occurs via a hydroxylamine formation which further suggested the possibility of successful biocatalytic substitution of second step of synthesis. *E. coli* efficiently reduced the precursor to D-DIBOA but the yield of the process remained ineffective. It was assumed that nitroreductases presented in strains are most likely responsible for reducing the precursor. Future experiments aim at optimization of variables and identification of enzymes responsible for reduction.

Gastrodin (Fig. 7) is the major constituent of the herb *Gastrodia elata Blume*. This plant has various clinical application such as sedative, anesthetic, memory enhancement, neuroprotection, anticonvulsant and free radical scavenging activities have been reported [62–66]. Zhang et al. [67] have demonstrated a simple microbial bioconversion of p-2-hydroxybenzyl alcohol (HBA) to gastrodin which can replace the synthetic technique.

Preliminary screening demonstrated *Armillaria luteo-virens* Sacc gave excellent transformation rate of HBA. This study took into account various parameters which optimized the gastrodin production. Maintenance of substrate concentration and inoculums size and addition of Tween 80 and oleic acid facilitated in achieving maximal gastrodin concentrations for 5 days. This simple novel biotransformation method could be scaled up conveniently and can also be applied for C-11 hydroxylation of steroids or related compounds [67].

Abel et al. [68] have reported the N- and O- demethylation of a thebaine derivative which yielded 3 potential buprenorphine intermediates. N- and O-demethylation via chemical route requires expensive and deleterious reagents and gives poor yields. Fungi of *Cunninghamella* species gave significant results. Fur-

thermore, process optimization and product characterization has improved the yield of N-demethylated compound from 39 to 94%. Two biotransformation pathways have been recognized which include a major one resulting in N-demethylated product and a minor one resulting in formation of N,O-didemethylated product. N-demethylation occurred more prominently at 32°C while N,O-demethylation was found at 28°C. The reason assumed was decreased methyl transferase activity. Cytochrome P450 was deduced to be responsible for N-demethylation as its inhibition by 1-aminobenzotriazole and metyrapone completely inhibited the reaction [69].

Ezetimibe is a potent inhibitor of biliary and dietary cholesterol absorption from small intestine but it does not affect absorption of bile acids, glycerol, and fat-soluble vitamins. As a matter of fact, its exact mechanism of action is yet to be discovered [70]. It has been suggested that it exerts effects through inhibition of acyl-coenzyme A: cholesterol acyltransferase resulting in decreased low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) production. Chiral alcohols serve as critical building blocks to synthesize pharmaceuticals demonstrating high enantiomeric purity [71]. In order to achieve economical, effective and conveniently scalable techniques for production of chiral pharmaceutical intermediates, enantioselective bioreduction has acquired significance [72]. Optimized reaction of 1-(4-fluorophenyl)-5-(2-oxo-4-phenyl-oxazolidin-3-yl)-pentane-1,5-dione (FOP dione) (Fig. 8) to give 3-[5-(4-fluorophenyl)-5-(S)-hydroxypentanoyl]-4(S)-4-phenyl-1,3-oxazolidin-2-one (FOP alcohol), a vital intermediate needed for ezetimibe synthesis produced excellent enantioselectivity and the higher yield due to oxidoreductase activity improvement [73]. It was observed that fructose when used as a carbon source maximized enzyme activity up to $[(5.07 \pm 0.028) \, \mu mol/min \, g \times 5 \times 10^{-3}]$ and cell mass up to (8.01 ± 0.14) g/L. Organic nitrogen sources supported cell growth but inorganic sources had no effect. Copper, zinc and potassium ions inhibited enzyme activity but 1.2 mM of Fe^{+3} was optimum for enzyme induction. Optimum bioreduction was achieved at pH 8.0, 40°C, a cell concentration of 250 mg/mL
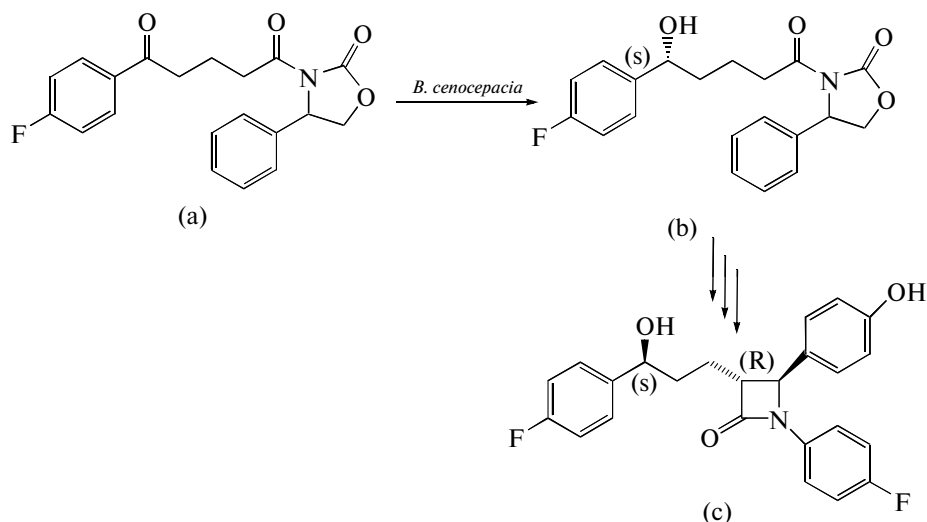
Fig. 8. Structures of 1-(4-fluorophenyl)-5-(2-oxo-4-phenyl-oxazolidin-3-yl)-pentane-1,5-dione (FOP dione) (a), 3-[5-(4-flu-orophenyl)-5(S)-hydroxypentanoyl-4(S)-4-phenyl-1,3-oxazolidin-2-one (b) and ezetimibe (c).

and agitation rate of 200 rpm. 54% chirally pure alcohol with >99% enantioselectivity was yielded which established *Burkholderia cenocepacia* as a harmless, efficient and potential biocatalytic substitute to hazardous and toxic Pd/C catalyst used for FOP dione reduction.

Alternatively, Kyslíková et al. [74] have analyzed the stereoselective reduction capacity of ketoreductase positive microbes using a carboxybenzyl protected form and unprotected form of the ketone 1-(4-uorophenyl)-3(R)-(3-oxo-3-(4-uorophenyl)-propyl)-4(S)-(4-hydroxyphenyl)azetidin-2-one (ezetimibe). Initial microbial screening of 230 microbes revealed *Rhodococcus fascians* MO22 had significant reduction capacities. Further optimization of the reaction parameters using above mentioned microorganism displayed that at pH 7.0 and 30°C using glucose or glycerol (50 g/L) as an enzyme inducer reduced 95% of the unprotected ketone and 63% of protected ketone with 20% suspension of cells.

RESOLUTION OF RACEMIC MIXTURE TO OBTAIN ENANTIOMERS POSSESSING DIFFERENT PHARMACOLOGICAL PROFILES

Optically pure single enantiomers cause less side effects as compared to their corresponding racemates [75]. Henceforth, the aim to design drugs with high enantiopurity and their corresponding unique activity profiles by means of racemate resolution has gained momentum [76]. Biocatalysts are imperative to achieve this objective as they surpass chemical catalysts once again for achieving the higher enantiomeric ratio (E) and enantiomeric excess (e.e) values.

Commercial enzyme preparations though offer an effective substitute, it often becomes difficult to obtain enzyme preparations possessing both enantioselectivity and kinetic resolution activity for racemates that are unnatural substrates. Lipases have been broadly used in enantioselective hydrolysis reactions to obtain the desired optically active acids or alcohols. Esterases contrarily inspite of their great biocatalytic capacity have been less used.

Ketoprofen which belongs to 2-arylpropionic acids is a racemic mixture. It inhibits prostaglandin synthesis and is used clinically as an anti-inflammatory and an analgesic compound. Its anti-inflammatory action is regarded to its S-enantiomer. Nevertheless, R-enantiomer has shown to possess some analgesic and antipyretic activity [77]. Gong et al. [78] have prepared R-enantiomer using racemic 2-ethyl ester of (R)-ketoprofen-2-(3-benzoylphenyl)propionic acid as substrate for *Citeromyces matriensis* CGMCC 0573. Careful microbial screening exhibited that most organisms had a natural tendency towards hydrolysis of S-enantiomer or they had a nonselective hydrolytic approach towards ketoprofen ester whereas microbes containing R-isomer hydrolyzing esterases were rare. Optimized conditions were maintained to enhace expression of esterase activity. Carbon sources had no effect but yeast extract as a nitrogen source proved effective. Isopropanol significantly reduced enzyme activity. The limitation of poor water solubility of ketoprofen was overcome by subsequent addition of ethanol and Tween 80 alternatively as this strategy eradicated the microbes using additives instead of substrate as an energy source. Tween 80 was assumed to contribute greatly to the ester hydrolysis by increasing membrane permeability of the yeast cells, inducing enzyme biosynthesis, providing carbon source for cell growth. Thus, Tween 80 enhances reaction rates. Characterization of esterase of *C. matriensis* CGMCC 0573 and its function regarding production
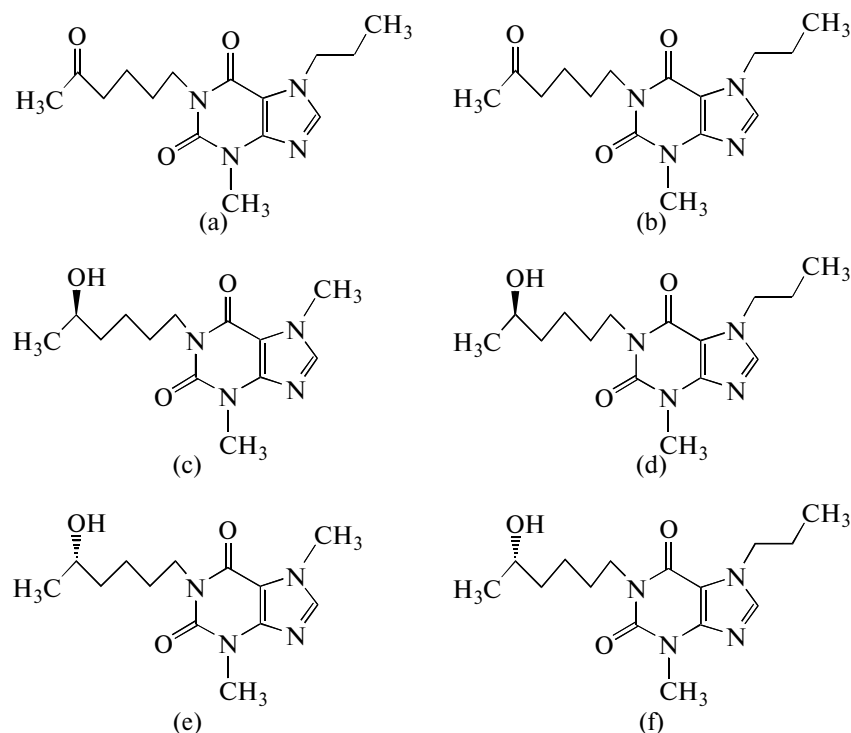
Fig. 9. Structures of pentoxifylline (PTX) (a), propentofylline (PPT) (b), lisofylline (LSF-(R)-OHPTX) (c), hydroxypro-pentofylline (1-(5-hydroxyhexyl)-3-methyl-7-propyl-xanthine) (R) − OHPPT (d), hydroxypentoxifylline (1-(5-hydroxyhexyl)-3,7-dimethyl-xanthine) (S) − OHPTX (e) and hydroxypropentofylline (1-(5-hydroxyhexyl)-3-methyl-7-propyl-xanthine) (S) − OHPPT (f).

of R-ketoprofen are underway by the authors. Liu et al. [79] have focused on clinical importance of S-keto-profen and directed their approach in developing a convenient method to obtain S-isomer by the mutant strain *Trichosporon laibacchii*. Racemic ketoprofen ester is hydrolyzed to yield a biotransformed broth consisting of ketoprofen acid considerably supplemented with S-isomer and ketoprofen ester considerably supplemented with R-isomer. The remaining ketoprofen ester after the biotransformation can be promptly puried, racemized and recycled to be used in further biotransformations to diminish raw-material costs. Procedure of optimization afforded to get satisfactory results in which E was 82.5 with an e.e. of 0.94. The two different procedures of biocatalytic resolution of ketoprofen ester are a vivid exemplification of stereospecificities of enzymes which in turn depend on their specific type or nature and direct their preference towards the formation of particular enantiomer from a racemic mixture.

Ibuprofen is a significant member of NSAIDS belonging to the 2-arylpropionic acids (profens family). Its anti-inflammatory activity is also believed to reside in S (+)-enantiomer. Microbial lipases have a great prospective for commercial uses because of their stability, enantioselectivity and broad substrate specicity. Chiral resolution of racemic ibuprofen via lipases is documented. Lipase from *Aspergillus niger* AC-54 spe-

cifically esterifies R (−)-ibuprofen and that afforded the best results in terms of enantioselectivity and thermostability as compared to other native lipases [80,81]. Carvalho et al. [82] have studied the characterization of parameters effecting the enantioselective resolution of (R,S)-ibuprofen by this lipase and optimized the technique of obtaining a cost-effective enzymic esterication. Variables influencing the control of the resolution of (R,S)-ibuprofen by lipase *A. niger* (such as enzyme concentration and ratio molar propanol : ibuprofen) were assessed. This experimental study provided a great contrivance to optimize the esterication conditions that allow an essential development of the enantiomeric excess of active (S)-ibuprofen and enantioselectivity of lipase in this process. Under optimum conditions, a fine enantioselective resolution of (R,S)-ibuprofen has been attained, which is significantly advanced previously reported results using this lipase.

β-blockers are drugs that antagonize the effects of catecholamines on β_1 and β_2 receptors. Atenolol and propranolol (Prop) are the most commonly used β-blockers which are used as racemic mixtures for their basic clinical effect i.e. hypertension. The biological activity of β-blockers resides mostly in S-enantiomer. Damle et al. [83] have successfully achieved high e.e. values for S-isomer of both atenolol and Prop employing resolution capacities of *Rhizopus ar-*

rhizus and *Geotrichum candidum*. Comparative studies performed showed that incubation of pure racemate of β-blockers furnished better results than corresponding esters of β-blockers. Thus, the superiority of the microbial transformation over other biological models (enzymes, mammals) was demonstrated in terms of cost-effectiveness, less time consumption and single step synthesis.

Endophytic fungi are a remarkable source for microbial biotransformation but they have been less investigated. The term endophytic fungus has been applied to those fungi which can be noticed at a specific moment in the tissues of apparently healthy plant host [84]. Kinetics of Prop transformation by some endophytic fungi has been recently evaluated which monitored metabolite concentrations as well as formation and consumption of metabolites corresponding to time. All endophytic fungi utilized had the ability to enantioselectively biotransform Prop to the active metabolite 4-OH-Prop. *Glomerella cingulata* showed significance for the production of the active enantiomer of the metabolite by transforming (−)-(S)-Prop to (−)-(S)-4-OH-Prop within 24 and 72 h of incubation. (+)-(R)-4-OH-Prop metabolite was formed after 72 h. *Aspergillus fumigatus* and *Chaetomium globosum* showed higher enantioselectivity in the production of the (−)-(S)-metabolite after 144 h of incubation [85]. These fungi have also been employed to biotransform enantioselectively thioridazine which is a phenothiazine neuroleptic used for psychiatric disorders such as schizophrenia. It is commercially available as a racemic mixture of (−)-(S) and (+)-(R)-enantiomers. *In vivo* experiments demonstrated sulfated metabolites to be pharmacologically active which are generated via oxidation of sulfur at 2-position producing thioridazine-2-sulfoxide (THD-2-SO). This metabolite undergoes additional oxidation to become sulfone THD-2-SO$_2$. Cardiotoxic effect of thioridazine is attributed to its metabolite THD-5-SO. The fungi used oxidized thiomethyl substituent at position 2 and sulfur at position 5 of phenothiazine ring. *Diaporthe phaseolorum* biotransformed (S)- and (R)-THD in relative amounts. The 2-sulfoxidation occurred with higher preference resulting in the R configuration of the sulfoxide to form (S,R)-THD-2-SO and (R,R)-THD-2-SO. Among all fungi evaluated, *A. fumigatus* exhibited higher formation of (S,S)-THD-2-SO and (R,R)-THD-2-SO metabolites. The 5-sulfoxidation in the thiazine ring was less [86].

Buspirone is an anxiolytic and antidepressant which most probably exerts its effects after binding to 5HT1A receptor. Liver cytochrome P450 3A4 metabolizes the drug generating its major metabolite 6-hydroxybuspirone which is present at 30−40 times higher concentration in human blood and thus it may be responsible for the clinical actions of the drug. Both the (R)- and (S)-enantiomers of 6 hydroxybuspirone separated by chiral HPLC, showed considerable potency in tests using a rat model of anxiety [87, 88]. While the (R)-enantiomer demonstrated rather strong binding and specicity for the 5HT1A receptor [89], the (S)-enantiomer had the benefit of being cleared more slowly from the blood [90]. Hanson et al. [89] have reported hydroxylation of buspirone to (S)-6-hydroxybuspirone directly by means *Streptomyces antibioticus* ATCC 14980. (S)-6-Hydroxybuspirone was also afforded enzymatically by the enantioselective hydrolysis of racemic 6-acetoxybuspirone using L-amino acid acylase. Hydrolysis of the isolated (R)-acetate yielded (R)-6-hydroxybuspirone.

Pentoxifylline (PTX: 1-(5-oxohexyl)-3,7-dimethyl-xanthine) and propentofylline (PPT: 1-(5-oxohexyl)-3-methyl-7-propyl-xanthine) are derivatives of theobromine which inhibit phosphodiesterase (Fig. 9). These drugs inhibit the transcription of the gene responsible for tumor necrosis factor (TNF-α) synthesis. As nonspecic phosphodiesterase inhibitors, they enhance the cAMP level in the cells, by this means inhibiting the synthesis of TFN-a, IL-1b, IL-6 and IL-8 [90, 91].

Hydroxypentoxifylline (OHPTX:1-(5-hydroxy-hexyl)-3,7-dimethyl-xanthine) and hydroxypropentofylline (OHPPT:1-(5-hydroxyhexyl)-3-methyl-7-propyl-xanthine) are pharmacologically important derivatives. The (R)-enantiomer of the OHPTX active metabolite, called as lisofylline (LSF), is a lysophosphatidic acid acyl-transferase inhibitor recognized as a drug candidate for the prevention of treatment-related toxicity in cancer patients [92, 93] and bone marrow transplant recipients [94]. Racemic OHPPT increases cerebral blood ow, but the (R)-enantiomer of OHPPT is three times more effective compared to the S-enantiomer in stimulating cerebral blood flow [95]. Pekala et al. [96] used *C. echinulata* NRRL1384 to biocatalyse the (S)-oxidation of the racemic hydroxy metabolites OHPTX and OHPPT and for (S)-reduction of PTX and PPT. The biotransformation of (±)-OHPTX gave an (R)-enantiomer (LSF) with an enantiopurity of approximately 93% e.e. compared to the bioconversion of (±)-OHPPT, where the greatest e.e. value for (R)-OHPPT was confirmed at 83%. Augmenting the bioconversion with glucose gave 47−51% yields and 80−93% e.e. values analogous to those obtained with no glucose.

* * *

Microbial biotransformation as a fundamental process has gained significant momentum whether it is concerning correlation studies with other *in vitro* (animal tissue, plant tissue, etc.) and *in vivo* (animals, humans) models or discovering metabolites superior from their predecessors in terms of activity, toxicity, pharmacokinetic and physicochemical parameters. However, no *in vitro* model could ever totally replace *in vivo* models as their predictive values are often questionable due to differing enzymology, physiology, reaction conditions etc.

Various drugs are transformed to active metabolites that may considerably participate in displaying overall pharmacology or adverse effects. They may inherit the exact pharmacological behavior with the added benefit of better safety and pharmacokinetic profile. On the contrary, they may exhibit pharmacological pattern differing significantly from the parent molecule. Active metabolites can be subjected to further structural modifications resulting in optimization of properties of parent molecule. The present review has shed light upon 3 important routes for designing therapeutic molecules. The numerous examples given above lead us to the conclusion that microbial biotransformation can prove to be an ideal tool for drug designing as it is cost effective, less hazardous environment friendly and easily scalable for metabolite production.

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