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REVIEW ARTICLE

A Perspective on Monoamine Oxidase Enzyme as Drug Target: Challenges and Opportunities

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Abstract: The monoamine oxidase (MAO) enzyme is responsible for the deamination of monoamine neurotransmitters and regulates their concentration in the central and peripheral nervous systems. Imbalance in the concentration of neurotransmitters in the brain and central nervous system is linked with the biochemical pathology of various neurogenic disorders. Irreversible MAO inhibitors were the first line drugs developed for the management of severe depression but most of these were withdrawn from the clinical practice due to their fatal side effects including food-drug interactions. New generations of MAO inhibitors were developed which were reversible and selective for one of the enzyme isoform and showed improved pharmacological profile. The discovery of crystal structure of MAO-A & MAO-B isoforms helped in understanding the drug-receptor interactions at the molecular level and designing of ligands with selectivity for either of the isoforms. The current article provides an overview on the MAO enzyme as potential drug target for different disease states. The article describes catalytic mechanism of MAO enzyme, crystal structures of the two MAO isoforms, traditional MAO inhibitors and various problems associated with their use, new developments in the MAO inhibitors and their potential as therapeutic agents especially in neurological disorders.

Keywords: Monoamine oxidase, neurological disorders, parkinson's disease, alzheimer's disease, depression, food drug interactions.

1. INTRODUCTION

Monoamine oxidase (MAO, EC 1.4.3.4) is a flavin adenine dineucleotide dependent enzyme. It is responsible for the deamination of monoamine neurotransmitters, endogenous amines and xenobiotics in the central and peripheral nervous systems [1]. In most of the mammal tissues, MAO enzyme is localised in the outer membrane of the mitochondria. The MAO enzyme has two isoforms, MAO-A and MAO-B where in MAO-A isoform is predominant in liver, gut, skin and placenta while MAO-B is the major isoform in the brain. Human MAO-A and MAO-B isoforms contain amino acid sequences that are 70% identical, however, differ in tissue distribution, substrate specificity and inhibitor selectivity. MAO-A has substrate specificity for the bulkier endogenous amines like serotonin, epinephrine and norepinephrine whereas MAO-B has substrate specificity for the small exogenous amines such as benzylamine, β-phenyl ethylamine [2]. Dopamine and tyramine are common substrates for both the isoforms. MAO enzyme can modulate the concentration of monoamine neurotransmitters in the central nervous system (CNS) and is linked with the etiology of number of neurological disorders. MAO enzyme is one of the extensively studied targets for the neurological disorders with more than 21000 publications recorded in the PubMed database.

2. CRYSTAL STRUCTURES OF MAO-A & MAO-B

The MAO enzyme is bounded covalently to co-factor FAD at cysteine residue by an 8-alpha (s-cysteinyl) riboflavin linkage with highly conserved structure [3]. The X-ray crystal structure studies revealed the existence of human MAO-A as monomeric and human MAO-B as dimeric units. Most of the membrane proteins exist in dimeric form [4] however through molecular docking studies and species-dependent genetic analysis [5] it has been concluded that the monomeric structure of human MAO-A could be due to Glu151Lys mutation near the dimer interface. The MAO-A and MAO-B isoform have cavities with volumes of 400 A^{o3} and ~ 700 A^{o3} respectively. The MAO-B cavity is divided into two parts *i.e.* entrance cavity of volume 290 A^{o3} and substrate cavity of volume ~400A^{o3} [6, 7]. Ile199 and Tyr326 side chains separate the two cavities in MAO-B isoform. The difference in the structure of active site of human MAO-A and MAO-B isoforms is due to the change in 7 out of 20 amino acid residues that line the active site of the enzyme and a change in the cavity shaping loop 210-216 [8]. In general the cavities of both the isoforms are hydrophobic in nature however MAO-B isoform contains a small highly con-

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served hydrophilic area in the entrance cavity. The structural comparison of the hMAO-A and hMAO-B could provide the molecular foundation for the design of highly specific reversible inhibitors for each of the enzyme isoform [9].

3. CATALYTIC ACTION OF MAO ENZYME

The MAO enzyme metabolizes amines to corresponding aldehydes, free amines and generates hydroxyl radicals that are highly toxic causing neuronal damage and death [10, 11]. The MAO catalysed reaction completes in two half-reactions, reduction half reaction and oxidation half reaction. In the reductive half-reaction, amine substrates are first oxidized by the covalent flavin cofactor to produce an imine intermediate, and flavin cofactor is reduced to hydroquinone. The reduced flavin is then re-oxidized by O_2 in the oxidative half-reaction to form H_2O_2. The H_2O molecule non-enzymatically hydrolyses the dissociated imine product to corresponding aldehyde and NH_4^+ as shown in Fig. (**1**) [12].

MAO mediated metabolism of monoamines is the major source of H_2O_2 production in the brain [13, 14]. In general the excess H_2O_2 is inactivated by the glutathione peroxidase (GPO) however in the presence of Fe^{2+} ions (Fenton reaction), it is converted into a highly reactive hydroxyl free radicals. The hydroxyl radicals are the main cause of oxidative stress and are believed to be responsible for the neuronal damage and death [13]. Thus, inhibition of MAO enzyme attenuates the oxidative deamination processes resulting in the reduced levels of H_2O_2 and hydroxyl free radicals which in turn provides relieves from oxidative stress (Fig. **2**).

4. ROLE OF MAO ENZYME IN NEUROLOGICAL DISORDERS

The MAO enzyme is reported to alter the level of neurotransmitters in the CNS. An imbalance in the concentration of neurotransmitters is linked with the biochemical pathology of various neurogenic disorders including depression, Alzheimer's disease (AD) and Parkinson's disease (PD) [15]. Different studies have proved that the over-activation of MAO enzyme in the brain plays a vital role in different psychiatric disorders. The elevated level of hMAO-B in the brain of AD patients is linked with the loss of cognitive functions [16, 17]. Similarly a number of research reports have suggested that the depletion of monoamine neurotransmitters concentration in severe depression cases could be attributed to the increase in hMAO-A levels in the cortex [18, 19].

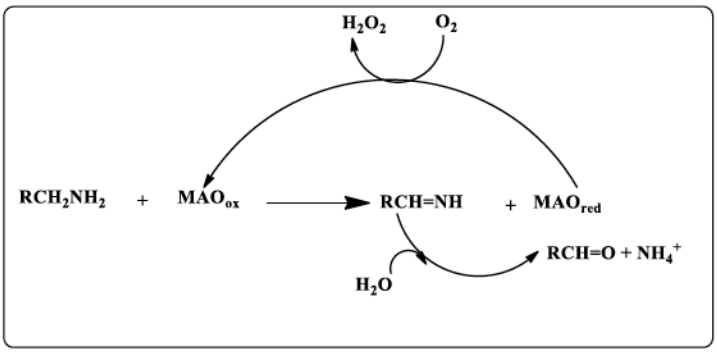

Fig. (1). General mechanism of catalytic action of MAO enzyme. Monoamines/neurotransmitters are oxidised by the MAO enzyme to imines which are further hydrolysed to corresponding aldehydes and ammonia by the addition of water molecule. The reduced flavin cofactor in MAO enzyme oxidizes to original form using oxygen (O_2) and this whole cycle generates a hydrogen peroxide (H_2O_2) molecule.

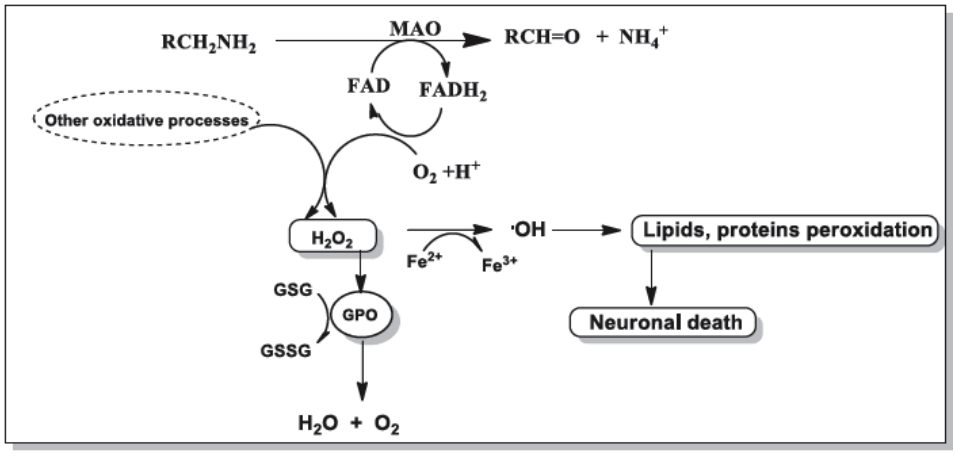

Fig. (2). Fenton reaction during oxidation cycle of MAO enzyme. Oxidation of FAD cofactor produces hydrogen peroxide (H_2O_2) which further generates reactive oxygen species leading to neuronal cell deaths.

4.1. Depression

Depression is a chronic condition and etiology of the disease is linked to the suboptimal concentration of monoamine neurotransmitters, serotonin and norepinephrine in the CNS [20]. The symptoms of depression include social anxiety disorder, severe phobias, posttraumatic stress disorder, and obsessive-compulsive disorder. The overexpression of MAO-A enzyme in the brain causes under-activity of monoamines such as dopamine, serotonin and norepinephrine that may lead to depression [21] (Fig. **3**). It has been observed that an antihypertensive drug reserpine decreases the storage levels of dopamine, serotonin and norepinephrine and low concentration of these neurotransmitters in the brain is linked to the depression [22]. Therefore, antidepressant therapy involves the maintenance of neurotransmitter level either by inhibiting MAO enzyme or blocking the presynaptic reuptake of serotonin, dopamine and norepinephrine. Hence, MAO enzyme has been recognized as an important drug target for the treatment/management of depression and a variety of structurally different ligands have been developed that can inhibit the MAO enzyme. Of late, selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) were introduced as anti-depressant agents for blocking the presynaptic reuptake of neurotransmitters.

4.2. Parkinson's Disease

MAO-B is the predominant isoform in the brain and its expression in the glial cell can increase up to four-fold with aging. Parkinson's disease (PD) is associated with the affected basal ganglia region where MAO-B isoform seems to be significantly responsible for the metabolism of dopamine [23]. Therefore, inhibitors of MAO-B isoform have been used in the management and treatment of PD. The Parkinson's disease is also linked with the exogenous toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The MAO-B enzyme converts MPTP to MPP+, a toxin responsible for the destruction of nigrostriatal dopaminergic neurons [23] (Fig. **4**). Thus, MAO-B inhibitors can prevent the damage of nigrostriatal neurons by halting the enzyme-mediated biotransformation of MPTP [24].

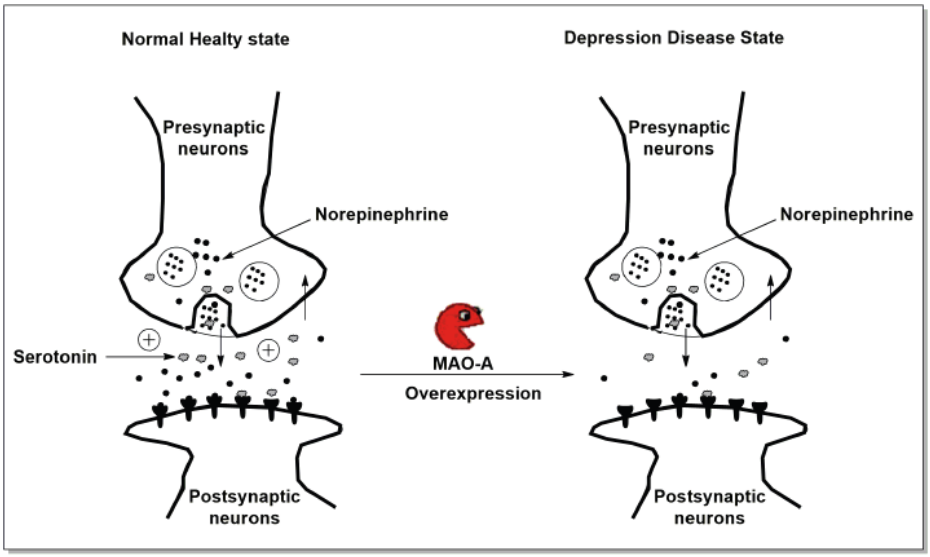

Fig. (3). General flow chart for the progression of depression. Over-expressed MAO-A isoform in the brain degrades serotonin and norepinephrine leading to the reduction in their concentration in the synaptic cleft and alteration in the proper emotional responses.

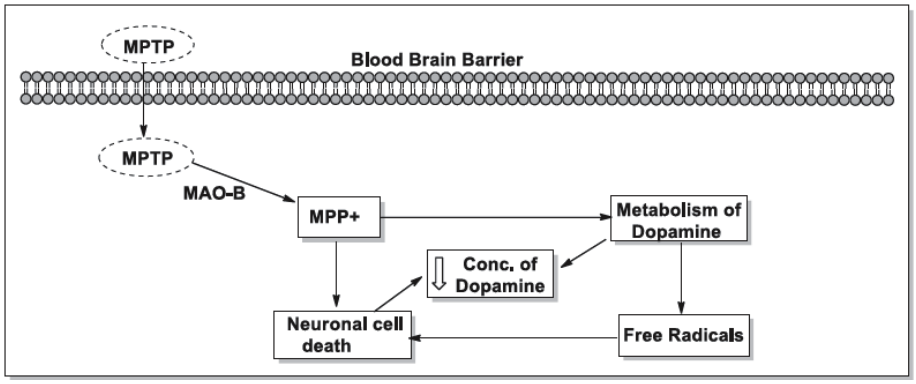

Fig. (4). General flow chart for the progression of Parkinson's disease. MPTP crosses blood brain barrier and converts to MPP+ in the presence of MAO-B. It causes neuronal cell death and increases the metabolism of dopamine in the brain. Metabolism of dopamine also generates free radicals that can result in neuronal cell deaths.

In the Parkinson's disease therapy, MAO-B inhibitors are combined with the levodopa, a precursor of dopamine, to maintain the concentration of dopamine in the CNS. The inhibition of the MAO-B enzyme stops the catalytic oxidation of dopamine in the brain and helps in maintaining the dopamine levels. This may help in the reduction of levodopa doses required for a therapeutic effect in the combination therapy. In addition to this, the MAO enzyme through oxidative deamination reaction, acts as a major source of hydrogen peroxide/hydroxyl free radicals in cells that may cause oxidative stress. Inhibition of the MAO-B isoform can reduce the levels of neurotoxic hydroxyl free radicals and suppresses many neurodegenerative processes associated with the PD [25].

4.3. Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder associated with the intellectual memory loss [26] and other cognitive impairments [27]. The exact cause of progressive neuronal degeneration in AD is not known, however etiology of the disease is linked with the presence of oxidative stress, and MAO-B hyperactivity in gliosis which increases the levels of H_2O_2 and oxidative free radicals [28]. MAO is a major enzyme responsible for the oxidative deamination of biogenic and xenobiotic monoamines. It generates hydrogen peroxide and hydroxyl free radicals that play a vital role in the progression of AD. In addition, low levels of acetylcholine were found in the specific brain regions of the AD patients that mediate learning and memory functions. Therefore, various acetylcholinesterase inhibitors (AChEIs) like tacrine, rivastigmine, donepezil, *etc*. have been developed for the treatment of AD. In the preclinical studies, it has been found that MAO-B inhibition can cause cognitive improvement and hence dual inhibitors targeting MAO-B and acetylcholinesterases can play an important role in the management/treatment of AD [29].

5. MAO INHIBITORS

The discovery of MAO inhibitors was a serendipitous finding. It was found that the patients suffering with tuberculosis and depression when treated with an anti-tuberculosis drug isoniazid, most of them showed improvement for depression during the treatment. Later on the anti-depression effect of isoniazid was attributed to its MAO inhibition potential. It was hypothesized that the deficiency in the concentration of neurotransmitters like dopamine, serotonin and norepinephrine in the brain may be responsible for the depression and other neurological disorders. Mechanistically MAO inhibitor binds with the enzyme and stops its catalytic action of monoamine oxidation and hence increases the concentration of neurotransmitters at the nerve terminals. The improved levels of these neurotransmitters assist in the symptomatic treatment of neurogenic disorders. The first generation MAO inhibitors were of irreversible nature and were predominantly used for the treatment of depression but their use diminished in the following years due to their undesired side effects and fatal drug-food interactions [30]. Various irreversible MAO inhibitors are described in Fig. (**6**).

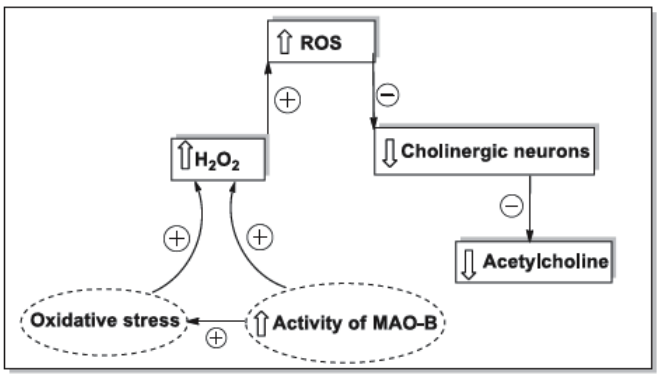

Fig. (5). General flow chart for the progression of Alzheimer's disease. The hyper-activity of MAO-B isoform in the brain increases the concentration of hydrogen peroxide and reactive oxygen species (ROS). This causes degradation of the cholinergic neurons and low levels of acetylcholine affects learning and memory functions of the brain.

6. PROBLEMS ASSOCIATED WITH THE TRADITIONAL MAO INHIBITORS

The first generation irreversible MAO inhibitors bind with the MAO enzymes making them permanently dysfunctional. The catalytic function of the enzyme could only be revived with the synthesis of new enzymes. The clinical use of irreversible MAO inhibitors caused high toxicity and other severe side effects including drug-food interactions, hepatotoxicity, hypertensive crisis, cheese effect, serotonin syndrome *etc*. [31]. Therefore, use of MAO inhibitors was abandoned and research focus was shifted towards other alternatives like serotonin selective reuptake inhibitors (SSRI) and serotonin norepinephrine reuptake inhibitors. However, MAO inhibitors were the only effective medication available for the treatment of severe and resistant depression.

6.1. Cheese Effect

Cheese effect is caused by the increased concentration of food tyramine in the blood due to inhibition of MAO-A isoform in the intestine. In general, the dietary tyramine is metabolized by the gut MAO-A and prevented from entering into the blood stream. In the presence of an irreversible MAO-A inhibitor, the food tyramine is not metabolised and its absorption increases in the blood. The excess amount of tyramine in the blood, initiates the release of adrenaline from peripheral adrenergic neurons and it may lead to severe increase in the blood pressure, termed as hypertensive crisis [32] (Fig. **7**). The food-drug interaction of irreversible MAO inhibitors was one of the major reasons for abandoning their clinical use in the treatment of depression.

6.2. Drug-Food Interactions

Consumption of number of foods, mainly cheese, by the patients taking MAO inhibitors may result in fatal hypertensive crises. The clinical syndrome associated with the hypertensive crisis is described as a sudden onset of a severe, palpitations, diaphoresis, stiff neck, pulsating headache, and nausea [33, 34]. Most common drug-food interactions in case of MAO inhibitors are with cheese due to the presence of various amines predominantly tyramine that causes hyper-

tensive crisis [35] also known as cheese effect. Another serious food interactions are with yeast products like beer, wine (presence of DOPA) that causes hypertension [36] and central adrenergic symptoms, with coffee, cola drinks (presence of Caffeine) to cause hyper excitability [37]. Other food contents that should be avoided along with MAO inhibitors includes marmite concentrated yeast, sauerkraut, soy sauce, broad bean pods, banana peels [38].

6.3. Drug-drug Interactions

MAO inhibitors can interact with various drugs and these interactions can be incredibly dangerous and potentially lethal. In general, there are two types of drug interactions with the MAO inhibitors. The first one is caused by serotonin reuptake inhibitory action known as serotonin syndrome and the second type of interactions can raise the blood pressure [31]. When MAO inhibitors are administered along with the drugs that increase adrenergic activity, a fatal hypertensive reaction may occur. For example, MAO inhibitors can interact with many of the decongestants (selective α_1 agonists) and raise the blood pressure [39]. MAO inhibitors should also be avoided with tricyclic antidepressants, analgesics and appetite suppressants that are norepinephrine reuptake inhibitors [40]. The combined effect of both the drugs may potentiate sudden increase of norepinephrine concentration in the blood which may lead to the hypertensive reaction. In addition to this, the use of MAO targeting antibiotics like linezolid along with MAO inhibitors can also initiate serious side effects [41]. Tramadol, mepridine and sympathomimetic drugs are some other medications which should be avoided along with the MAO inhibitors [42]. Drug-drug interactions between the potential antidepressants like moclobemide–fluoxetine and amitriptyline–diazepam can also lead to severe hepatic toxicity [43]. Combination of MAO inhibitors with the barbiturates cause prolonged sedation while their combination with the phenothiazines can cause increased extrapyramidal reactions. MAO inhibitors along with the pethidine can causes rigidity and coma within minutes after injection [44]. Other sympathomimetic and stimulant drugs that should be avoided with MAO inhibitors include ephedrine, phenylephedrine, amphetamines and cocaine.

6.4. Serotonin Syndrome

Serotonin is a neurotransmitter playing an active role in a number of physiological functions. Serotonin is one of the important targets for anti-depression therapy and in case of severe depression, its concentration in the brain and CNS is maintained by the combined use of MAO inhibitors and selective serotonin reuptake inhibitors. However, simultaneous inhibition of MAO and serotonin transporter can increase the concentration of serotonin in the synaptic cleft [45]. The over stimulation of serotonin receptors can cause disruption in the thermoregulation that may lead to fatal hyperthermia termed as 'serotonin syndrome' [46]. Dextromethorphan (over-the-counter drug) medication along with MAO inhibitors is associated with the serotonin syndrome [47].

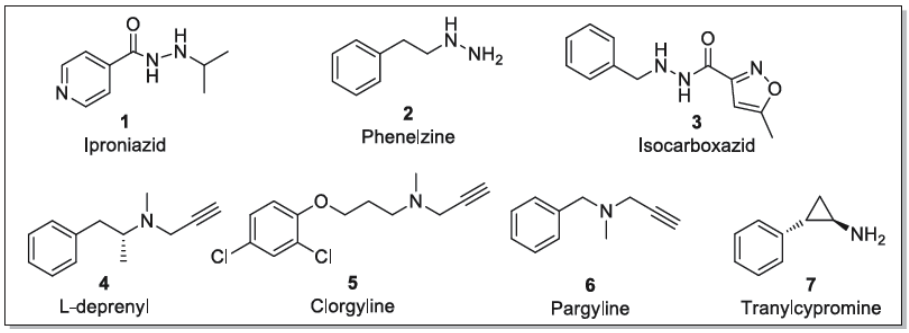

Fig. (6). Irreversible MAO inhibitors used for the treatment of depression and other neurological disorders.

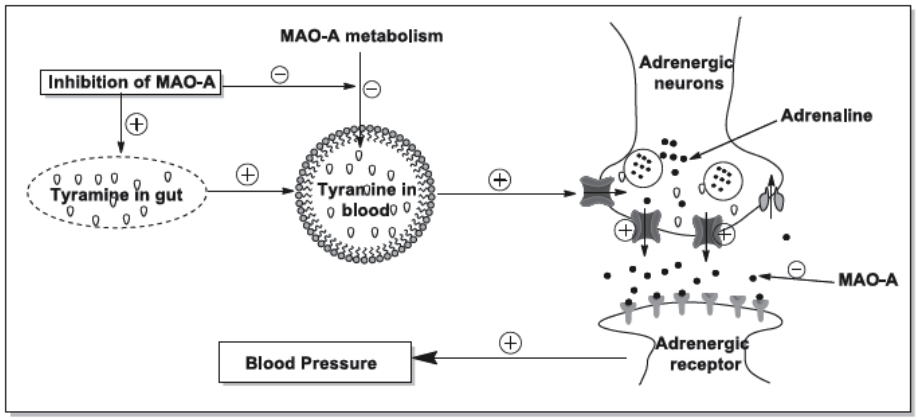

Fig. (7). Cheese effect: Irreversible inhibition of MAO-A isoform leads to the increased concentration of tyramine in the gut. The increased concentration of the tyramine initiates the release of adrenaline from the adrenergic neurons that may result in increased blood pressure or severe hypertension.

7. ADVANCEMENTS IN MAO INHIBITORS

The irreversible MAO inhibitors were responsible for many side effects including severe drug-food and drug-drug interactions as described above. Use of irreversible inhibitors can lead to permanent deactivation of MAO enzyme that might disrupt many of the enzyme mediated physiological processes and generally enzyme activities could only be revived with the synthesis of new enzyme. Hence, most of the irreversible MAO inhibitors were discontinued from the clinical practice.

7.1. Reversible and Selective MAO Inhibitors

New generation of MAO inhibitors were developed which were not only reversible in nature but also selective for one of the MAO isoforms. The inhibitors exhibiting selectivity for the MAO-A isoform have the potential to be developed as drugs for the treatment of depression and anxiety disorders, [48] and MAO-B selective inhibitors can be effective in several neurogenic disorders such as PD, AD, Huntington chorea and amyotrophic lateral sclerosis [49]. The main advantage of developing reversible MAO inhibitors was the absence of 'Cheese effect' [50, 51]. The reversible and selective inhibitors bind to the MAO-A isoform and inhibit the enzyme with decreased risk of tyramine reaction. The absorbed tyramine releases norepinephrine which competes with the reversible MAO-A inhibitors and reactivates MAO-A enzyme in the intestine, liver and sympathomimetic neurons. Reversible and MAO-A selective inhibitors like moclobemide **(12)** (Fig. **8**), toloxatone **(18)** (Fig. **9**), *etc*. were found effective in the treatment of depression [52]. Examples of some reversible MAO-A inhibitors under clinical practice include moclobemide, toloxatone, pirlindole, befloxatone and brofaromine.

In addition, MAO-A selective inhibitors were found to play crucial role in the treatment of anxiety disorders and atypical depression. MAO-B selective inhibitors do not potentiate the cheese reaction until administered at concentrations high enough to inhibit MAO-A isoform. Reversible and MAO-B selective inhibitors like farnesol (19) and rasagiline **(20)** (Fig. **10**) were found effective in PD, and their neuroprotective properties in animal models suggested their potential role in the treatment of AD.

The X-ray crystal structures of MAO-A and MAO-B isoforms with their selective inhibitors like harmine **(15)** (Fig. **9**) and farnesol **(19)** (Fig. **10**) respectively are available. The docking studies of these selective inhibitors provided crucial information about the nature of the active site of the enzyme and binding mode of ligands. Structure based drug designing is a useful strategy for the discovery of more potent and selective MAO inhibitors that can be developed as potent drug candidates. A large number of MAO inhibitors with structurally different scaffolds including pyrazoles [53], coumarins [54, 55], indoles [56, 57], imidazole [58], oxazolidinone

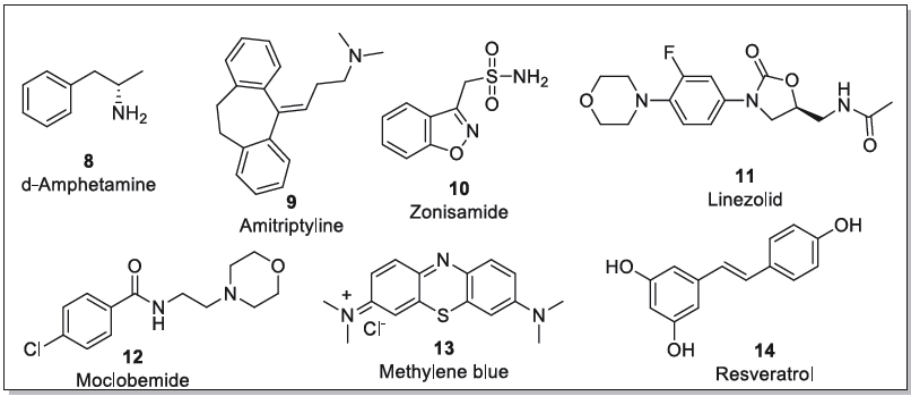

Fig. (8). Reversible MAO inhibitors developed to negate various side effects of first generation irreversible MAO inhibitors.

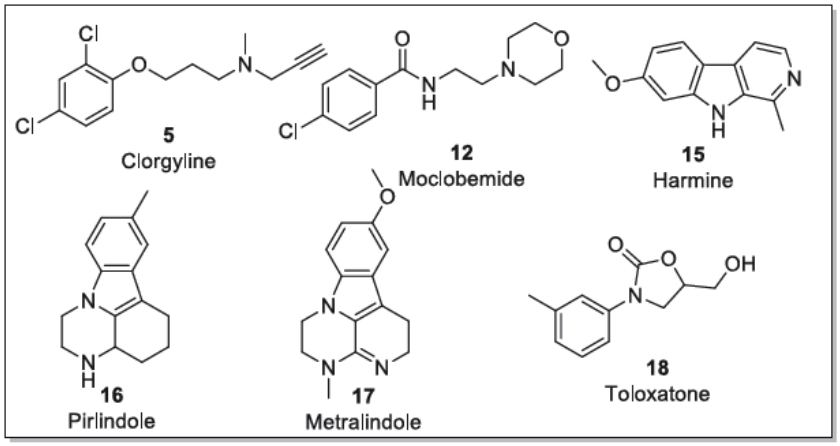

Fig. (9). Some representative molecules developed as selective MAO-A inhibitors.

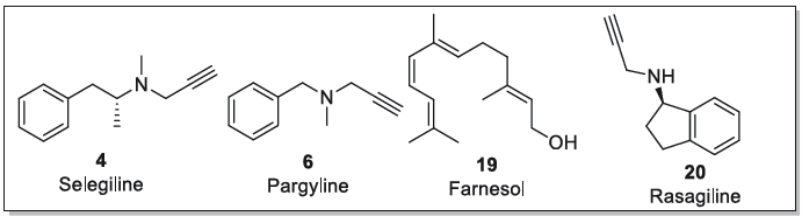

Fig. (10). Some representative molecules developed as MAO-B selective inhibitors.

[59], alkaloids [60] *etc*. have been synthesized in order to develop potent and effective drug candidates for the treatment of various neurogenic disorders [61]. Similarly, a number of compounds isolated from natural sources and belonging to different chemical classes like flavonoids, xanthones, alkaloids, cannabinoids, proanthocyanidins, iridoidglucosides, curcumin *etc*. have been screened for their MAO inhibition potential [62].

7.2. Transdermal Delivery System

Transdermal systems have been developed for the direct delivery of MAO inhibitors into blood circulation avoiding first pass through the liver. Transdermally, the drug crosses blood-brain barrier in a high concentration and eliminates the risk of cheese effect [63] in the gut. This inhibits both MAO-A and MAO-B in the brain but avoids drug interactions with MAO enzyme in the gut. These new developments reduce the risk of hypertensive crisis making MAO inhibitors comparatively safer therapeutic agents. Selegiline was the first MAO inhibitor delivered through transdermal patch system [64]. Transdermal system delivers the drug over an extended period of time and for low doses of inhibitors no dietary restrictions are required [15]. Rasagiline has been also formulated in a transdermal patch [65] in order to overcome its drawbacks like limited solubility and patient's compliance. Rasagiline is marketed as a rasagiline mesylate salt to be taken orally once in a day. As most of the PD patients have difficulties in oral administration of the drug, a transdermal formulation was desirable for a less-frequent administration. To increase the penetration rate of the drug into the skin, rasagiline was used as a free base which was soluble in the lipophilic polymer matrix and the patch was covered with an inert backing layer that regulated the drug release. The prototypes were submitted to several skin permeation experiments and revealed an excellent permeation rate.

8. NOVEL MAO INHIBITORS UNDER CLINICAL TRIALS

MAO enzyme has been explored as an important target for the development of potent and effective drug candidate for the treatment of various neurological disorders. A large number of scaffolds belonging to different chemical classes have been screened for their MAO inhibition potential. The extensive screening efforts have led to the identification of some lead compounds that showed high potency and selectivity for either of the MAO isoform. For example, Karuppasamy *et al.* [66] screened a series of 3,5-diarylpyrazoline derivatives and identified compound **21** as lead compound with an IC_{50} value of 150±10.05 nM and selectivity index of greater than 10000 towards MAO-A isoform. Abdelhafez *et al.* [67] screened a number of oxadiazole, thiadiazole, triazole and thiazolidinone derivatives of 4-methyl and 3,4-dimethyl-7-oxycoumarins and reported compound **22** and compound **23** as highly potent leads with *Ki* of 5.01 pM & 5.18 pM, and selectivity index of 9.66×10^4 and 9.58×10^4 towards MAO-A respectively. Through structure-activity relationship studies, Khattab *et al.* [68] identified compound **24** as the most promising lead with an IC_{50} value of 2.8 ± 0.13 nM and selectivity index of 3×10^6. Valente *et al.* [59] reported compound **25**, a 3-(1*H*-pyrrol-3-yl)-2-oxazolidinone derivative, as a reversible and potent MAO-A inhibitor with a selectivity index of 2 X 10^5. Similarly, some highly potent and MAO-B selective inhibitors have been identified with the help of biological screening and through various structure-activity relationship studies. Secci *et al.* [69] screened a series of arylidene-(4-substituted-thiazol-2-yl)hydrazines and identified compound **26** as a selective MAO-B inhibitor with an IC_{50} value of 2.54 ± 0.17 nM and selectivity ratio more than 39,000 (MAO-B). Recently, Mertens *et al.* [55] reported 3-(4-methoxy)phenyl derivative of alkynyl-coumarinyl ethers (compound **27**) as a selective inhibitor of MAO-B isoform with an IC_{50} value of 2.96 ± 0.10 nM and more than 3400 folds selectivity over MAO-A isoform. Two different series of compounds, based on chromane-2,4-dione and chromone-3-carboxamide scaffolds, were screened and the compound **28** was reported as an important lead with an IC_{50} value of 2.9 nM and selectivity index of more than 3400 folds [70]. Walt *et al.* [71] evaluated sulfanylphthalonitrile and sulfanylbenzonitriles analogous as MAO inhibitors and reported compound **29** as the most promising MAO-B inhibitor (IC_{50} = 25 nM) with very high selectivity (8720-fold). Tzvetkov *et al.* [72] reported *N*-(3,4-difluorophenyl-1*H*-indazole-5-carboxamide (compound **30**) with remarkable *in vitro* MAO-B inhibitory potential (IC_{50} = 1.59 nM and SI more than 6000 fold) and a balanced physicochemical profile for the CNS bioavailability. Many of the promising lead compounds are undergoing toxicological studies and are in various stages of clinical developments.

Safinamide, a reversible MAO-B selective inhibitor, has completed Phase III trials for the treatment of PD. Safinamide was found to be an effective inhibitor of dopamine uptake and a modulator of glutamate release [73]. Varinel Inc., a US based pharmaceutical company, is developing VAR-10200 **(31)** and VAR-10300 **(32)** (Fig. **11**) as dual iron chelating and MAO-B selective inhibitors for the treatment of neurodegenerative disorders (Thomson Reuters Pharma, update of Feb. 24, 2012 and May 15, 2012) [74]. Ladostigil (TV-3326) **(33)** is being developed by Avraham Pharmaceutical as dual MAO and acetylcholinesterase inhibitor. This compound is under phase II clinical trials in Europe (Thomson Reuters Pharma, update of May 18, 2012). Orizon Genomics is developing OG-45 as a dual inhibitor of MAO-B and lysine specific demethy-

lase-I (Thomson Reuters Pharma, update of April 16, 2012). Similarly, Roche is developing RG1577 (RO-4602522; EVT-302), as a lead compound for the treatment of AD. This orally active compound is reversible and selective inhibitor of MAO-B isoform and is under phase II clinical trials (Thomson Reuters Pharma, update of August 8, 2012). Rasagiline **(20)** (Fig. **11**) rescue with clinicalTrials.gov identifier no NCT02359552 is under phase II clinical trial from Feburary 2015 for the treatment of AD. The aim of the study is to evaluate the effect of the drug in patients suffering from moderate AD. Rasagiline (Azilect) with clinicalTrials.gov identifier no NCT02068625 is under phase 4 of clinical studies from February 2014 to investigate its effects in patients suffering from macula off retinal detachment.

Currently, CX157 (3-fluoro-7-(2,2,2-trifluoroethoxy) phenoxathiin-10,10-dioxide), a selective MAO-A inhibitor, is under clinical development for the treatment of major depressive disorders [75].

CONCLUSION AND FUTURE PERSPECTIVE

The MAO enzyme plays crucial role in the regulation of various monoamines and neurotransmitters in peripheral and central nervous system. The MAO catalysed metabolism of monoamines produces hydroxyl free radicals causing neural damage and death. The over expression of MAO enzyme is linked with the pathology of many neurogenic disorders. The MAO enzyme has been recognised as an important drug target and MAO inhibitors were developed as drug candidates for the treatment of diseases like depression, PD and AD. First generation MAO inhibitors were irreversible and non-selective in nature and showed severe drug-food interactions and other side effects. Consequently, new types of reversible and selective MAO inhibitors were developed to address various problems associated with the first generation inhibitors. Reversible MAO-A inhibitors are comparatively safer and are replaced by noradrenalin in the gut to reactivate the enzyme while MAO-B selective inhibitors are devoid of 'cheese reaction'.

Both the isoforms of MAO enzyme share a high sequence similarity and it is very difficult to generalize structure based selectivity of ligands for MAO-A or MAO-B isoform. The discovery of crystal structures of human MAO-A and MAO-B isoforms with their respective ligands, helped in the structure based designing of ligands for these enzyme isoforms. The MAO-A cavity is bigger in size and accommodates bulkier groups while the entrance cavity of MAO-B is smaller in size and comprises small hydrophilic pocket and hence shows preference for smaller and hydrophilic groups. Considerable progress has been made to understand the ligand interactions with the two enzyme isoforms and their proximity to the FAD cofactor. Molecular modeling studies provide crucial information about the orientation of ligand in the active site and its interaction. Information about ligand-enzyme binding interactions may be beneficial in the development of potent and effective drug candidates for various neurological disorders.

Recently, MAO inhibitors have been proposed as neuroprotecting and neurorescue agents [76]. The neurotoxic aldehyde and hydroxyl free radical generated during MAO catalysed metabolism of monoamine neurotransmitters are responsible for the neural damage and death. The inhibition of MAO enzyme halts the catalytic cycle and decreases the production of hydroxyl free radicals. The MAO-B isoform is also involved in the metabolism of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to the neurotoxin MPP+ (1-methyl-4-phenylpyridinium) which is involved in the inhibition of mitochondrial electron transport and increases ROS production. The elevated ROS production is responsible for several neurodegenerative disorders and MAO-B inhibitors stop both MPTP metabolism as well as the resulting damage to the neurons. The underlying mechanism of neurorescue and neuroprotective potential of MAO inhibitors is not clear due to the conflicting evidence obtained through clinical studies [77, 78] and further research work needs to be done to establish the protective mechanisms.

Although many newly developed reversible and selective MAO inhibitors [79] are in clinical practice but most of these

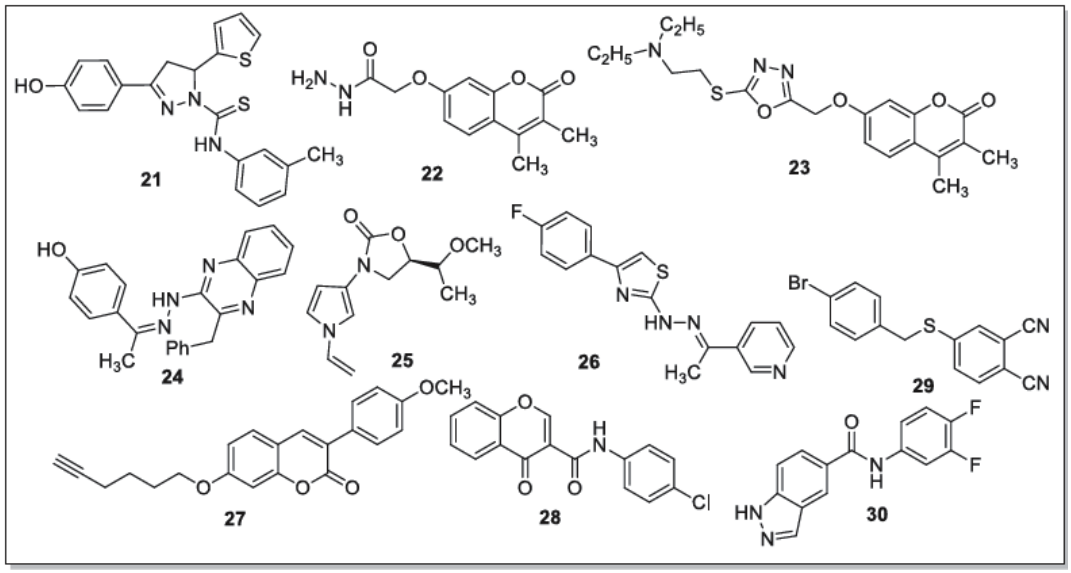

Fig. (11). Some recently developed lead compounds as a selective MAO-A (21-25) and MAO-B (26-30) inhibitors.

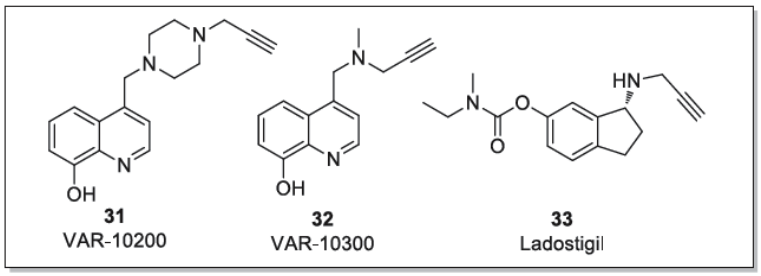

Fig. (12). MAO inhibitors under clinical development.

are providing symptomatic relief. The underlying mechanism and etiology of various neurological diseases and their progression is still a mystery. The MAO enzyme plays a very complex role in the regulation of neurotransmitters and it is still not clear how different MAO activities are controlled in the brain. The neurogenic disorders are of complex nature and it is difficult to manage these through the single target like MAO. Most of the neurogenic disorders originate through multiple pathogenic factors and hence the one-drug-one-target strategy has limited success with the MAO inhibitors. Multi-target based drug discovery is the alternative approach in which a single compound can bind to multiple targets so as to manage multiple symptoms. Organic chemistry and Computational chemistry are the tools to design ligands with features for selective inhibition of MAO and other enzyme such as cholinesterases. A lot of research work still needs to be done in order to establish the exact role of MAO inhibitors in the etiology of other disorders like ageing, cerebral ischaemia, neuroprotection/neurorescue and substance-abuse risk.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Legoabe LJ, Petzer A, Petzer JP. Inhibition of monoamine oxidase by selected C6-substituted chromone derivatives. Eur J Med Chem 2012; 49: 343-53.

[2] Juarez-Jimenez J, Mendes E, Galdeano C, *et al.* Exploring the structural basis of the selective inhibition of monoamine oxidase A by dicarbonitrile aminoheterocycles: Role of Asn181 and Ile335 validated by spectroscopic and computational studies. Biochim Biophys Acta 2014; 1844(2): 389-97.

[3] Santana L, Uriarte E, Gonzalez-Diaz H, Zagotto G, Soto-Otero R, Mendez-Alvarez E. A QSAR model for *in silico* screening of MAO-A inhibitors. Prediction, synthesis, and biological assay of novel coumarins. J Med Chem 2006; 49(3): 1149-56.

[4] Sachs JN, Engelman DM. Introduction to the membrane protein reviews: the interplay of structure, dynamics, and environment in membrane protein function. Annu Rev Biochem 2006; 75: 707-12.

[5] Andres AM, Soldevila M, Navarro A, Kidd KK, Oliva B, Bertranpetit J. Positive selection in MAOA gene is human exclusive: determination of the putative amino acid change selected in the human lineage. Hum Genet 2004; 115(5): 377-86.

[6] Binda C, Wang JP, Leonardo, Caccia CC, Angelo, Salvati PE, Dale E Mattevi, Andrea. Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: safinamide and coumarin analogs. J Med Chem 2007; 50(23): 5848-52.

[7] Milczek EM, Binda C, Rovida S, Mattevi A, Edmondson DE. The 'gating'residues Ile199 and Tyr326 in human monoamine oxidase B function in substrate and inhibitor recognition. FEBS J 2011; 278(24): 4860-69.

[8] Hubálek F, Binda C, Khalil A, Li M, Mattevi A, Castagnoli N, Edmondson DE. Demonstration of isoleucine 199 as a structural determinant for the selective inhibition of human monoamine oxidase B by specific reversible inhibitors. J Biol Chem 2005; 280(16): 15761-66.

[9] De Colibus L, Li M, Binda C, Lustig A, Edmondson DE, Mattevi A. Three-dimensional structure of human monoamine oxidase A (MAO A): relation to the structures of rat MAO A and human MAO B. Proc Natl Acad Sci USA 2005; 102(36): 12684-89.

[10] Inaba-Hasegawa K, Akao Y, Maruyama W, Naoi M. Rasagiline and selegiline, inhibitors of type B monoamine oxidase, induce type A monoamine oxidase in human SH-SY5Y cells. J Neural Transm 2013; 120(3): 435-44.

[11] Riederer P, Sofic E, Rausch WD, Schmidt B, Reynolds GP, Jellinger K, Youdim MB. Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. J Neurochem 1989; 52(2): 515-20.

[12] Edmondson DE, Bhattacharyya AK, Walker MC. Spectral and kinetic studies of imine product formation in the oxidation of p-(N, N-dimethylamino) benzylamine analogs by monoamine oxidase B. Biochemistry 1993; 32(19): 5196-202.

[13] Salach JI, Weyler W. Preparation of the flavin-containing aromatic amine oxidases of human placenta and beef liver. Method Enzymol 987; 142: 627-37.

[14] Pizzinat N, Copin N, Vindis C, Parini A, Cambon C. Reactive oxygen species production by monoamine oxidases in intact cells. Naunyn Schmiedebergs Arch Pharmacol 1999; 359(5): 428-31.

[15] Wimbiscus M, Kostenko O, Malone D. MAO inhibitors: risks, benefits, and lore. Clev Clin J Med 2010; 77(12): 859-82.

[16] Kennedy B, Ziegler M, Alford M, Hansen L, Thal L, Masliah E. Early and persistent alterations in prefrontal cortex MAO A and B in Alzheimer's disease. J Neural Transm 2003; 110(7): 789-801.

[17] Anand R, Wesnes K. Cognition-enhancing effects of moclobemide, a reversible MAO inhibitor, in humans. Adv Neurol 1990; 51: 261-8.

[18] Benedetti MS, Dostert P. Monoamine oxidase, brain ageing and degenerative diseases. Biochem Pharmacol 1989; 38(4): 555-61.

[19] Emilsson L, Saetre P, Balciuniene J, Castensson A, Cairns N, Jazin EE. Increased monoamine oxidase messenger RNA expression levels in frontal cortex of Alzheimer's disease patients. Neurosci Lett 2002; 326(1): 56-60.

[20] Leysen D, Pinder RM. . Toward third generation antidepressants. Annu Rep Med Chem 1994; 29: 1-12.

[21] Kabayama M, Sakoori K, Yamada K, *et al.* Rines E3 ubiquitin ligase regulates MAO-A levels and emotional responses. J Neurosci 2013; 33(32): 12940-53.

[22] Brigitta B. Pathophysiology of depression and mechanisms of treatment. Dialogues Clin Neurosci 2002; 4(1): 7-20.

[23] Coelho Cerqueira E, Netz PA, Diniz C, Petry do Canto V, Follmer C. Molecular insights into human monoamine oxidase (MAO) inhibition by 1, 4-naphthoquinone: Evidences for menadione (vitamin K3) acting as a competitive and reversible inhibitor of MAO. Bioorg Med Chem 2011; 19(24): 7416-24.

[24] Perez V, Unzeta M. PF 9601N [*N*-(2-propynyl)-2-(5-benzyloxy-indolyl) methylamine], a new MAO-B inhibitor, attenuates MPTP-induced depletion of striatal dopamine levels in C57/BL6 mice. Neurochem Int 2003; 42(3): 221-9.

[25] Manley-King CI, Bergh JJ, Petzer JP. Inhibition of monoamine oxidase by selected C5-and C6-substituted isatin analogues. Bioorg Med Chem 2011; 19(1): 261-74.

[26] Samadi A, de los Ríos C, Bolea I, *et al.* Multipotent MAO and cholinesterase inhibitors for the treatment of Alzheimer's disease: Synthesis, pharmacological analysis and molecular modeling of heterocyclic substituted alkyl and cycloalkyl propargyl amine. Eur J Med Chem 2012; 52: 251-62.

[27] Corey-Bloom J, Thal L. Monoamine oxidase inhibitors in Alzheimer's disease. Neurol Dis Ther 1994; 21: 279-89.

[28] Christen Y. Oxidative stress and Alzheimer disease. Am J Clin Nutr 2000; 71(2): 621s-629s.

[29] Bortolato M, Chen K, Shih JC. Monoamine oxidase inactivation: from pathophysiology to therapeutics. Adv Drug Deliver Rev 2008; 60(13): 1527-33.

[30] Ramsay R. Inhibitor design for monoamine oxidases. Curr Pharm Design 2012; 19(14): 2529-39.

[31] Amsterdam JD, Shults J. MAOI efficacy and safety in advanced stage treatment-resistant depression - a retrospective study. J Affect Disord 2005; 89(1): 183-8.

[32] Youdim MB, Bakhle Y. Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. Brit J Pharmacol 2006; 147(S1): S287-S296.

[33] Fiedorowicz JG, Swartz KL. The role of monoamine oxidase inhibitors in current psychiatric practice. J Psychiatr Practice 2004; 10(4): 239-48.

[34] Blackwell B, Marley E, Price J, Taylor D. Hypertensive interactions between monoamine oxidase inhibitors and foodstuffs. Br J Psychiatry 1967; 113(497): 349-65.

[35] Blackwell B, Marley E, Ryle A. Hypertensive crisis associated with monoamine-oxidase inhibitors. Lancet 1964; 283(7335): 722-23.

[36] Hodge J, Nye E, Emerson G. Monoamine-oxidase inhibitors, broad beans, and hypertension. Lancet 1964; 283(7342): 1108.

[37] Kline NS. Psychopharmaceuticals: effects and side effects. Bull World Health Organ 1959; 21(4-5): 397-410.

[38] Sweet RA, Brown EJ, Heimberg RG, *et al.* Monoamine oxidase inhibitor dietary restrictions: what are we asking patients to give up? J Clin Psychiatry 1995; 56(5): 196-201.

[39] Stahl SM, Felker A. Monoamine oxidase inhibitors: a modern guide to an unrequited class of antidepressants. CNS Spectr 2008; 13(10): 855-71.

[40] Ciraulo DA, Shader RI. Fluoxetine drug-drug interactions: I. Antidepressants and antipsychotics. J Clin Psychopharmacol 1990; 10(1): 48-50.

[41] Lorenz RA, Vandenberg AM, Canepa EA. Serotonergic antidepressants and linezolid: a retrospective chart review and presentation of cases. Int J Psychiat Med 2008; 38(1): 81-90.

[42] Zetin M. A Clinician's Guide to Monoamine Oxidase Inhibitors. Current Psychiatry Rev 2013; 9(4): 353-64.

[43] Voican CS, Corruble E, Naveau S, Perlemuter G. Antidepressant-induced liver injury: a review for clinicians. Ame J Psychiatry 2014; 171(4): 404-15.

[44] Sjöqvist F. Psychotropic drugs (2) Interaction between monoamine oxidase (MAO) inhibitors and other substances. Proc R Soc Med 1965; 58(11 Pt 2): 967-78.

[45] Birmes P, Coppin D, Schmitt L, Lauque D. Serotonin syndrome: a brief review. Can Med Assoc J 2003; 168(11): 1439-42.

[46] Boyer EW, Shannon M. The serotonin syndrome. New Engl J Med 2005; 352(11): 1112-20.

[47] Sternbach H. The serotonin syndrome. Am J Psychiatry 1991; 148(6): 705-13.

[48] Fuller RW. Selectivity among monoamine oxidase inhibitors and its possible importance for development of antidepressant drugs. Prog Neuro-Psychopha 1978; 2(3): 303-11.

[49] Yamada M, Yasuhara H. Clinical pharmacology of MAO inhibitors: safety and future. Neurotoxicology 2004; 25(1): 215-21.

[50] Rao TS, Yeragani VK. Hypertensive crisis and cheese. Ind J Psychiatry 2009; 51(1): 65.

[51] Finberg J, Gillman K. Selective inhibitors of monoamine oxidase type B and the “cheese effect”. Int Rev Neurobiol 2011; 100: 169-90.

[52] Youdim MB, Weinstock M. Therapeutic applications of selective and non-selective inhibitors of monoamine oxidase A and B that do not cause significant tyramine potentiation. Neurotoxicology 2004; 25(1): 243-50.

[53] Mathew B, Suresh J, Anbazhagan S, Elizabeth Mathew G. Pyrazoline: A promising scaffold for the inhibition of monoamine oxidase. Cent Nerv Syst Agents Med Chem 2013; 13(3): 195-206.

[54] Matos MJ, Vilar S, Gonzalez-Franco RM, *et al.* Novel (coumarin-3-yl) carbamates as selective MAO-B inhibitors: Synthesis, *in vitro* and *in vivo* assays, theoretical evaluation of ADME properties and docking study. Eur J Med Chem 2013; 63: 151-61.

[55] Mertens MD, Hinz S, Müller CE, Gütschow M. Alkynyl–coumarinyl ethers as MAO-B inhibitors. Bioorg Med Chem 2014; 22(6): 1916-28.

[56] Prins LH, Petzer JP, Malan SF. Inhibition of monoamine oxidase by indole and benzofuran derivatives. Eur J Med Chem 2010; 45(10): 4458-66.

[57] Patil PO, Bari SB, Firke SD, Deshmukh PK, Donda ST, Patil DA. A comprehensive review on synthesis and designing aspects of coumarin derivatives as monoamine oxidase inhibitors for depression and Alzheimer’s disease. Bioorg Med Chem 2013; 21(9): 2434-50.

[58] Villarinho JG, Fachinetto R, de Vargas Pinheiro F, *et al.* Antidepressant-like effect of the novel MAO inhibitor 2-(3, 4-dimethoxyphenyl)-4, 5-dihydro-1H-imidazole (2-DMPI) in mice. Prog Neuropsychopharmacol Biol Psychiatry 2012; 39(1): 31-39.

[59] Valente S, Tomassi S, Tempera G, Saccoccio S, Agostinelli E, Mai A. Novel Reversible Monoamine Oxidase A Inhibitors: Highly Potent and Selective 3-(1 H-Pyrrol-3-yl)-2-oxazolidinones. J Med Chem 2011; 54(23): 8228-32.

[60] Passos CS, Simoes-Pires CA, Nurisso A, *et al.* Indole alkaloids of *Psychotria* as multifunctional cholinesterases and monoamine oxidases inhibitors. Phytochemistry 2013; 86: 8-20.

[61] Patil PO, Bari SB. Nitrogen heterocycles as potential monoamine oxidase inhibitors: Synthetic aspects. Arab J Chem 2014; 7(1): 857-84.

[62] Carradori S, D’Ascenzio M, Chimenti P, Secci D, Bolasco A. Selective MAO-B inhibitors: a lesson from natural products. Mol Divers 2014; 18(1): 219-43.

[63] Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov 2004; 3(2): 115-24.

[64] Cascade EF, Kalali AH, Preskorn SH. Emsam: The first year. Psychiatry (Edgmont) 2007; 4(6): 19-21.

[65] Carradori S, Secci D, Bolasco A, Chimenti P, D'Ascenzio M. Patent-related survey on new monoamine oxidase inhibitors and their therapeutic potential. Expert Opin Ther Pat 2012; 22(7): 759-801.

[66] Karuppasamy M, Mahapatra M, Yabanoglu S, *et al.* Development of selective and reversible pyrazoline based MAO-A inhibitors: Synthesis, biological evaluation and docking studies. Bioorg Med Chem 2010; 18(5): 1875-81.

[67] Abdelhafez OM, Amin KM, Ali HI, Abdalla MM, Batran RZ. Synthesis of new 7-oxycoumarin derivatives as potent and selective monoamine oxidase A inhibitors. J Med Chem 2012; 55(23): 10424-36.

[68] Khattab SN, Hassan SY, Bekhit AA, El Massry AM, Langer V, Amer A. Synthesis of new series of quinoxaline based MAO-inhibitors and docking studies. Eur J Med Chem 2010; 45(10): 4479-89.

[69] Secci D, Bolasco A, Carradori S, D’Ascenzio M, Nescatelli R, Yanez M. Recent advances in the development of selective human MAO-B inhibitors: (Hetero) arylidene-(4-substituted-thiazol-2-yl) hydrazines. Eur J Med Chem 2012; 58: 405-17.

[70] Cagide F, Silva T, Reis J, Gaspar A, Borges F, Gomes L, Low J. Discovery of two new classes of potent monoamine oxidase-B inhibitors by tricky chemistry. Chem Commun 2015; 51(14): 2832-5.

[71] Van der Walt MM, Terre’Blanche G, Lourens AC, Petzer A, Petzer JP. Sulfanylphthalonitrile analogues as selective and potent inhibitors of monoamine oxidase B. Bioorg Med Chem Lett 2012; 22(24): 7367-70.

[72] Tzvetkov NT, Hinz S, Küppers P, Gastreich M, Müller CE. Indazole-and indole-5-carboxamides: Selective and reversible monoamine oxidase B inhibitors with subnanomolar potency. J Med Chem 2014; 57(15): 6679-703.

[73] Stocchi F, Vacca L, Grassini P, *et al.* Symptom relief in Parkinson disease by safinamide Biochemical and clinical evidence of efficacy beyond MAO-B inhibition. Neurology 2006; 67(7 suppl 2): S24-9.

[74] Zheng H, Weiner LM, Bar-Am O, *et al.* Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases. Bioorg Med Chem 2005; 13(3): 773-83.

[75] Burch D, Asgharnejad M, Gerson W, Fielding RM, Azzaro AJ. Lack of tyramine pressor response effect with oral CX157: A specific reversible MAOI. Clin Pharmacol Drug Dev 2014; 3(1): 4-12.

[76] Naoi M, Maruyama W. Functional mechanism of neuroprotection by inhibitors of type B monoamine oxidase in Parkinson's disease. Expert Rev Neurother 2009; 9(8): 1233-50.

[77] Pelusio M. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. New Engl J Med 1993; 328: 176-83.

[78] Group PS. Impact of deprenyl and tocopherol treatment on Parkinson's disease in DATATOP subjects not requiring levodopa. Ann Neurol 1996; 39: 29-36.

[79] Kumar B, Sheetal, Mantha AK, Kumar V. Recent developments on the structure-activity relationship studies of MAO inhibitors and their role in different neurological disorders. RSC Adv 2016: 42660-83.

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