ABSTRACT: Monoamine oxidase (MAO) is a protein with a key function in the catabolism of neuroamines in both central and peripheral parts of the body. MAO-A and -B are two isozymes of this enzyme which have emerged to be considered as a drug target for the treatment of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). Isatin is an endogenous small fragment, reversible inhibitor for MAO enzymes and is more selective for MAO-B than -A. Isatin is responsible for increasing the dopamine level in the brain by the inhibition of an MAO enzyme. The very few selective and reversible inhibitors existing for MAO proteins and the intensity of neurological diseases in humanity have opened a new door for researchers. Isatin has a polypharmacological profile in medicinal chemistry, is a reversible inhibitor for both the MAOs, and shows high selectivity potent inhibition for MAO-B. In this review, we discuss isatins and their analogues phthalide and phthalimide with structure−activity relationships (SARs), and this comprehensive information accelerates the ideas for design and development of a new class of MAO inhibitors for neurodegenerative diseases.

INTRODUCTION

Isatin (2,3-dioxindole) is a pharmacologically active agent, which is derived from the indole (Figure 1) nucleus, and it was discovered in 1841 by Erdman and Laurent. However, isatin is an endogenous small molecule in humans, which is widely distributed in the body fluid and different tissues of mammals and also naturally originates in the plants of the genus Isatis and in Boronia koniamboensis (New Caledonia), Melochia tomentosa (United States, Mexico), and Couropita guianensis Aubl (Central America and Amazon region) species. The Stolle, Gassman, Martinet, and Sandmeyer procedures were used to synthesize various isatin analogues by the conventional methods. Isatin is a highly reactive chemical that has been used as a nucleophile and an electrophile in synthetic chemistry. Nucleophilic additions to the third position keto group are the most well-known reaction of isatin as an electrophile. Spiro compounds have now been discovered to have significant pharmacological action, particularly in the science of organic products. Several investigations on the synthesis of spiro analogues of isatin at the C-3 position have been published. Meshram and colleagues described the synthesis of isatin derivatives containing the spiroxindole component via a three-component reaction comprising isatin, amino acids, and but-2-yneodioates in an aqueous medium employing microwave irradiation with base- and catalyst-free conditions. As a nucleophile, the reaction undergoes substitution at γ-lactam nitrogen and electrophilic substitution on the aromatic ring. Besides, derivatives of isatin have shown certain chemical reaction like ring expansion, oxidation, aldol condensation, and Friedel−Crafts reaction (Figure 1).

Isatin is an attractive oxidized indole nucleus and has received much attention as a framework in the design of numerous methods. Isatin is a highly reactive chemical that has been used as a nucleophile and an electrophile in synthetic chemistry. Nucleophilic additions to the third position keto group are the most well-known reaction of isatin as an electrophile. Spiro compounds have now been discovered to have significant pharmacological action, particularly in the science of organic products. Several investigations on the synthesis of spiro analogues of isatin at the C-3 position have been published. Meshram and colleagues described the synthesis of isatin derivatives containing the spiroxindole component via a three-component reaction comprising isatin, amino acids, and but-2-yneodioates in an aqueous medium employing microwave irradiation with base- and catalyst-free conditions. As a nucleophile, the reaction undergoes substitution at γ-lactam nitrogen and electrophilic substitution on the aromatic ring. Besides, derivatives of isatin have shown certain chemical reaction like ring expansion, oxidation, aldol condensation, and Friedel−Crafts reaction (Figure 1). Isatin is an attractive oxidized indole nucleus and has received much attention as a framework in the design of numerous methods.
molecules established as inhibitors of anticonvulsants, apoptosis, anxiolytics, and antifungal, antimalarial, potential antitumor, antibacterial, antitubercular, and antiviral agents. Many drugs have recently been reported for the treatment of renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumors like Sunitinib.

Glover et al., Clow et al., and Armando et al. found that tribulin, an endogenous displacer benzodiazepine and monoamine oxidase inhibitor (MAO), has been detected in humans and rats (brain and heart). The chemical composition of tribulin was unclear until Glower and colleagues discovered that isatin had features that are similar to endogenous tribulin. Isatin is identified as a potent inhibitor for MAO, and its selectivity to MAO-B is more than that to MAO-A.

MAOs are imperative enzymes that have been usually investigated for the treatment of neurological diseases such as Parkinson’s disease (PD) and Alzheimer’s disease (AD). The oxidative deamination of biogenic and xenobiotic amines is catalyzed by this enzyme, which changes their levels in the brain. Flavin-containing amine oxidoreductase is a protein family that catalyzes the breakdown of neurotransmitters. Two different types of MAOs, namely, MAO-A and MAO-B, both share ~70% sequence similarity and are found to originate in the CNS in humans, and both contain the cofactor flavin adenine dinucleotide (FAD). These enzymes break down the neuroamines, such as serotonin (5-HT), dopamine (DA), adrenaline, noradrenaline (NA), and phenylethylamine (PEA). MAO-A selectively deaminates 5-HT and noradrenaline (NE), and clorgyline inhibits it irreversibly. Selegiline, on the other hand, inhibits MAO-B, which selectively deaminates benzylamine and phenethylamine (PEA) in an irreversible manner. The newest marketed FDA-approved drug safinamide and lazabemide (nonmarketed) are reversible MAO-B inhibitors. Moreover, the isatin small molecule is identified as a reversible MAO enzyme. The PDB IDs 1OJA and 2XFP are two protein 3D structures in MAO-B and MAO-A, respectively. The crystal structure of 2XFP reveals that two fragments, compound 3 (2-BFI) and isatin, respectively, block the entrance and substrate-binding cavities. In the isatin complex, hydrophobic contacts were seen in Ile199, Ile198, and Leu171 as well as a hydrogen connection with the cofactor FAD via the water molecule. Two carbonyl groups compose a hydrophobic environment with Tyr326, Phe343, Leu171, and Tyr60.

Isatins have been used for PD treatment as a MAO-B inhibitor in the last two decades. Isatin is able to increase the dopamine level in striatum by inhibiting MAO-B. Previously, MAO-related quinazoline, chalcone, coumarin, and pyrazoline and the key role of halogen bonds have been established in previous reviews, but even though isatin is an established MAO inhibitor no reviews on an indole-based nucleus (isatin) have been established. Thus, we concentrate on this scaffold of an indole-based nucleus, and we have tried to explain the SAR study of isatin, phthalide, and phthalimide-based analogues.

Few isatin analogues were investigated by Medvedev et al., in vitro for their MAO-B and MAO-A inhibitory activity, and the majority of the analogues was found to be less effective than isatin. Substitutions on the fifth, sixth, and seventh positions of the aromatic ring of isatin seemed to increase its MAO inhibitory activity. The OH group in the fifth, sixth, and seventh positions showed increased activity when compared to methyl-substituted compounds. Isatinic acid which is obtained through the hydrolysis of isatin showed the highest activity. In comparison, hydroxylation of the aromatic ring’s fifth position showed higher selectivity for MAO-A than -B.

Structurally similar compounds of isatin were also taken into consideration, and it was found that these analogues also seemed to show potent MAO inhibitory activity. Medvedev et al. reported that apart from isatin structurally similar compounds like oxindole (1), indole (2), and dopamine-isatin (4) (Figure 3) were also evaluated. The IC$_{50}$ value of $N$-methyl isatin (5) was 7.9 ± 0.4 μM, and the IC$_{50}$ value of analogues of isatin and the hydroxylated form of isatin molecules was 14 ± 3 μM. Surprisingly, from the evaluation, we can clearly understand that 5-hydroxyisatin (6) showed the highest bioactivity against the MAO-A enzyme with an IC$_{50}$ value of 8.4 ± 1.4 μM, followed by unsubstituted isatin and the further remaining compounds.

From the above provided data, it is very clear that the introduction of an oxo group to the indole ring shows enhanced inhibitory potential toward MAO-B and not much toward MAO-A. Among the indole compound substituted with an oxo group in the second and third positions, the 3-oxo group containing an indole compound was shown to have the highest inhibitory potency when compared to that of the 2-oxo group containing an indole compound which may be due to the unreactiveness of the 2-oxo group, and the 3-oxo group forms a Schiff base with a free amino group of the MAO compound.

There are numerous compounds that have features similar to that of isatin structurally, such as indole (2), oxindole (1), and much more. The more detailed our analysis, the more we are able to explore the structural features of isatin analogues. Michelle et al. suggested that they have screened four compounds similar to isatin and 5-hydroxyisatin (6) and compared them with each other.
From the IC$_{50}$ values obtained, the compounds 5-hydroxyindole (3) showed more selectivity toward the MAO-A enzyme than MAO-B. Among the four analogues, we can see that the substitution of the OH group in the fifth position of the oxindole increased the selectivity toward MAO-A, whereas substitution of the OH group in the fifth position of isatin showed reduced selectivity toward MAO-A.

In the previously conducted studies, we can see that isatin was 3 times more effective toward MAO-B, while the 5-hydroxyisatin (6) which is the synthetic analogue of isatin was 3 times more effective toward MAO-B than MAO-A. Interestingly, while investigating the concentration of each compound in the brain, it became evident that the concentration of 5-hydroxyoxindole (3) is 2 times higher. While comparing the two synthetic analogues of oxindole (1) and isatin, it shows that 5-hydroxyoxindole (3) is a 23 times more effective inhibitor of MAO-A with an IC$_{50}$ value of $(8.4 \pm 1.4 \times 10^{-6} \, \mu$M) than 5-hydroxyisatin with an IC$_{50}$ value of $6.5 \pm 1.1 \, \mu$M. Overall, this SAR study concludes that a hydroxy group at the fifth position of isatin increases the selectivity toward the MAO-A. Additionally, C-3 and N-1 positions on the oxo and methyl group, respectively, increase affinity toward MAO-B (Figure 4).

Isatin, an indole-1-dione ring with a molecular weight of 147.13, has some intermediates that are called conjugated isatin anions, as reported by Berci-Filho et al.,$^{51}$ and it is very clearly understood that anions, because of their weakly bonded valence electrons, produce strong van der Waals interactions with the molecules surrounding them when compared to that of neutral molecules. In a study, Surendra et al.$^{52}$ explain the need for a coplanar structure of substituents at C-2 and C-3 for the selectivity toward MAO-A inhibition.

According to molecular docking studies with MAO-B, the capacity of the (E)-styrylisatin derivatives to connect the enzyme’s substrate cavity and the entrance cavity is the best explanation for the binding affinity of the (E)-styrylisatin derivatives in comparison to isatin. The worst binding of the derivatives to human MAO-B is supported by testing using the Ile199 Ala mutant.$^{53}$

A group of ten C5- and C6-substituted isatin derivatives (Figure 5) were synthesized in a work by Manley-King et al.$^{53,54}$ with the goal of determining their MAO inhibitory characteristics. This is based on the literature, which claims that reacting C3-substituted aniline derivatives with diethyl ketomalonate produces isatins with substitution in the C6 position. According to the selectivity index, isatin is 1.57 times more highly selective toward MAO-B than MAO-A. The result that molecule 7 is an effective inhibitor of MAO-B with IC$_{50}$ values of 0.009 $\mu$M was consistent with previous findings. The fact that the 4-phenylbutyl group is the longest side string studied in this review supports the notion that a longer side string improve isatin’s MAO-B inhibitory efficacy more than shorter side chains. The 4-phenylbutyl side string had no effect on isatin’s MAO-A inhibition potency, in contrast to its effect on MAO-B inhibition activity. Compound 8 with an IC$_{50}$ value of 2.19 $\mu$M only weakly inhibited MAO-A and was roughly 14 times more powerful than isatin’s MAO-A inhibitory strength. Likewise, the isatin derivatives that are substituted at C6 and C5 with the benzyl group were found to be efficient MAO-B inhibitors, with IC$_{50}$ values of 0.138 $\mu$M and 0.103 $\mu$M, respectively. Compounds 9 and 10 were poorer MAO-B inhibitors in comparison with the benzyl-substituted isatin counterparts, with IC$_{50}$ values of 9.93 $\mu$M and 1.40 $\mu$M, respectively. Compound 10 was 13 times less powerful than the equivalent isatin analogues which are C5-benzyloxy substituted, while compound 9 was 71 times less powerful than the corresponding isatin analogue with C6-benzyloxy substitution.

Figure 4. Structure—activity relationship of isatin derivatives.

![Figure 4](image)

Figure 5. Structure of styryl-based isatin derivatives.

![Figure 5](image)
As a result, we can state that the substitution of isatin at the C-5 position is preferable when compared to C-6 substitution for increasing isatin’s MAO inhibitory potencies. The fact that C-6 and C-5 substitution significantly improves isatin’s MAO-B inhibition effectiveness is consistent with the theory that the C-6 and C-5 side chains connect with the enzyme’s entrance cavity residues, which allows for more valuable interactions with the protein and thus more effective inhibition when compared to isatin.53

C-6- and C-5-substituted isatin derivatives (Figure 6a) are reversible inhibitors of hMAO-A and -B, according to the literature. Compared to isatin, C-6 and C-5 substitutions increase binding affinity to both MAO isoforms and, in most cases, preferential selectivity toward MAO-B isozymes. The isatin ring binds to the substrate cavities of MAO-A and MAO-B, according to modeling and crystallographic studies, and is stabilized by hydrogen bond connections between the C-2 carbonyl oxo and the NH of the dioxo indolyl scaffold and water compounds existing in the substrate cavities of MAO-B and MAO-A. The only isatin derivatives tested in this work that inhibited MAO-A potently were compound 29, with IC\textsubscript{50} values of 0.233 and 0.562 μM, respectively. With IC\textsubscript{50} values of 1.40 and 9.93 M, respectively, compounds 24 and 25 were mild MAO-B inhibitors compared to the benzyloxy replacement isatin derivatives (11, 12). Molecules 13 were about 13 times less powerful than the equivalent C-5 benzyloxy substituted isatin derivatives (11), while 14 were about 71 times less effective than the corresponding compound 12. Similar to the benzyloxy-substituted counterparts, compounds 15, 16, 17, and 18 were shown to be substantially poorer MAO-B inhibitors (11, 12). As a result, the phenyl, 2-phenylethyl, and phenoxy side strings do not boost the MAO-B binding attraction of isatin in the same way as the benzyloxy and (E)-styryl side strings. Compound 19 inhibited MAO-A only modestly, with an IC\textsubscript{50} value of 2.19 μM, almost 14 times more powerful than isatin at inhibiting MAO-A. In order to support their findings, they carried out molecular docking and molecular modeling studies for compounds 11. In contradiction to the interaction positions of isatin and 11 in the human MAO-B active site, the dioxoindolyl ring of 11 in the MAO-A active site is rotated through 180°. With an interplane distance of roughly 3.6 Å\textsuperscript{3}, the dioxoindolyl ring of 11 may form a π−π contact with the amide functional group of the Gln-215 side chain (Figure 6b). The isatin ring generates one extra hydrogen bond contact with the active site residues and fluids of MAO-A and -B compared to the aniline counterparts, according to molecular docking studies. This might explain why the isatin analogues have higher MAO inhibitory potencies than the comparable aniline counterparts. Possible π−π stacking interactions between the isatin ring and the amide p-face of an active site Gln residue may also help to stabilize the complexes between the isatin analogues and the MAO isozymes. Gln-215 forms stacking contacts with isatin 11 in MAO-A, whereas Gln-206 forms stacking interactions with 11 in MAO-B.54

This SAR study concludes that, as shown in Figure 7, the C-5 position of isatin in halogen substitution on benzyloxy increases the benzal chain and the affinity toward MAO-B. The C-6 position in benzyloxy increases the selectivity for MAO-B, and the phenyl substitution increases MAO-A inhibition. Based on these findings, as well as the structural similarities between phthalimide (isomer of isatin) and isatin, a range of

Figure 6. (a) Structures of benzyloxy- and benzal-based isatin derivatives. (b) Compound 11 with MAO-B interaction.

Figure 7. Structure−activity relationship for benzyloxy- and benzal-based isatin derivatives.
Phthalimide derivatives (Figure 8) have been created and tested as MAO inhibitors by Clarina et al. [2011]. Here, in this work, a group of C-5-substituted phthalimide compounds 21–31 were synthesized and tested for their inhibition of recombinant hMAO-B and -A. The findings showed that the compounds are extremely effective hMAO-B inhibitors with good reversibility with IC₅₀ values in the lower nanomolar range for the majority of them. The SAR study showed that lengthening of the C-5 substituent improves the phthalimide analogues’ MAO-B inhibitory potency. Halogen replacement on the ring system of the C-5 side strand can boost the efficacy of MAO-B inhibitors, especially the weaker phthalimide inhibitors.

Because N-substituted phthalimides (30 and 31) are poor MAO-B inhibitors, the location of the substituent at C-5 is structurally significant. The examination that phthalimide is indeed a poor MAO-B blocker adds to the evidence that the C-5 substituent is important for affinity to MAO-B. Modeling studies show that high affinity binding requires polar attractions between the polar area of the MAO-B substrate cavity and the phthalimide ring, as well as van der Waals forces between the entry cavity and the C-5 side string. The observation that halogen replacement on the aromatic ring of the C-5 side string boosts MAO-B inhibitor effectiveness supports the notion that interactions between the entry cavity and the C-5 side string are crucial for inhibitor binding. As a result of these structural modifications, the C-5 side string and the entry cavity are anticipated to have more successful van der Waals interactions. Because van der Waals attractions within the entry cavity most likely stabilize the phenyl ring of the C-5 side chain, halogen replacement on the phenyl ring (25–27) is bound to improve the inhibitor’s meaningful interactions with the MAO-B entry gate cavity via hydrophobic discretion and dipole connections.

The C-5 substitution of phthalimide derivatives 21–29 inhibited MAO-A in a reversible manner. The phthalimide derivatives are MAO-B-selective inhibitors, except from 5-phenoxophthalimide (21), which is seen to be nonselective. An increase in the MAO-A inhibitory activity takes place by the lengthening of the C-5 side chain, and the halogen substitution on the benzene ring of the C-5 side string increased the MAO-A inhibitory bioactivities of the phthalimide derivatives, but to a smaller amount than MAO-B. These findings imply that the C5 side chain interacts with the MAO-A active site in a productive manner. The fact that phthalimide is just a poor MAO-A inhibitor highlights the significance of the C-5 side string in MAO-A binding. As a result, although a substituent at the C5 position is essential for MAO-B inhibition, a substituent at the C5 position is necessary for phthalimide’s MAO-A inhibitory action. Finally, C-5-substituted phthalimides have high MAO-B binding attractions, making them good candidates for developing novel selective reversible MAO-B inhibitors. Furthermore, analogues like compound 26, which also have powerful MAO-A inhibitory properties, might be used as lead molecules in the development of nonselective reversible MAO-A and -B inhibitors. The results of C5-substituted phthalimide analogues 21–29 inhibiting hMAO-A and -B were revealed through the molecular modeling reports. It was detected that the C-5 substituent is crucial for the phthalimides’ MAO-B and MAO-A inhibiting activities. This is exhibited by the fact that N-substituted phthalimides and phthalimide (30 and 31) are poor MAO-B and MAO-A inhibitors that do not bind to the proteins in some situations, even at large doses. It was also noteworthy that among the phthalimides examined the phenylpropenylxy-substituted derivative 28 showed the lowest MAO-A inhibitory bioactivity while having a high MAO-B inhibitory activity. The molecular docking experiments were conducted using the Discovery Studio 1.7 modeling program, following a technique previously published. Despite this, the MAO-A active site has numerous polar functional groups that are within the hydrogen bond contact distance with compound 28. The o xo group at C-1 of the phthalimide ring may cause hydrogen interaction with an active site water compound, whereas the phthalimide NH proton may form hydrogen interaction with the phenolic hydrogen of Tyr444 and a water compound at C-1 of the phthalimide ring (HOH-739 and HOH-710). The extension of the C5 substituent by its length or size enhances the inhibitory potencies of the phthalimide analogues, which is clearly understood from the structure–activity relationship. Halogen replacement on the ring system of the C-5 side strand improves the efficiency of MAO-B inhibitors, especially for the weaker phthalimide inhibitors. Because N-substituted phthalimides are poor MAO-B inhibitors, the substitution at C-5 has structural significance. The fact that phthalimide is a poor MAO-B blocker provides more proof for the relevance of the C-5 substituent for interacting with MAO-B. Finally, C-5-substituted phthalimides have high MAO-B binding energies and are thus appropriate lead molecules for the expansion of new reversible selective MAO-B inhibitors. Furthermore, analogues like 25, which also have substantial MAO-A inhibition capabilities, might be used as lead molecules in the development of reversible nonselective MAO-A and -B inhibitors.

Figure 8. Structure of phthalimide-based derivative.
MAO inhibitors have been designed using a wide range of heterocyclic moieties. Isatin is a natural blocker of MAO-B and -A. Phthalimide, an isatin isomer, has now been classified as a possible scaffold for MAO-B-selective inhibitor development. Though phthalimide is a mild MAO inhibitor, C-5 substitution results in compounds that are very selective and effective MAO-B inhibitors. N-Substitution, on the other hand, results in molecules that are largely deprived of MAO inhibitory characteristics. By substituting on the C-8 locations of caffeine and the C-5 and C-6 locations of isatin, the MAO-B inhibitory profile of both can be improved. In this reference, the benzoxyl substituent appears to be particularly beneficial, and benzoxyl substitution of caffeine, isatin, and caffeine phthalimide yields compounds like 32, 33, and 34 (Figure 9) which are many times more effective MAO-B inhibitors than the core molecules.

With an interplane length of roughly 3.4, a putative pi–pi contact between the amide group of the amino acid Gln215 and phthalimide rings may also exist. The CS side chain of compound 28 is bent at a 50° angle from the caffeine ring plane, resulting in a bent conformation. In this SAR study (Figure 10), benzoxyl and the bridge of benzoxyl is a key role for MAO inhibition, and halogen substitution also affects the affinity. The N-position of phthalimide on substitution does not increase the MAO-B inhibition much.

Van der Walt et al. explained the binding of isatin analogues, based on which they further investigated (E)-styrlylsatin analogues for their MAO inhibitory activity. According to the 3D architecture of a hybrid between isatin and hMAO-B, isatin interacts with the substrate cavity via C-2 carbonyl oxo and dioxindolyl NH hydrogen linked to ordered water compounds in the active site. The MAO-B entry cavity is left empty as a result of this binding approach. According to these structural criteria, styril replacement at C-5 results in molecules that span both cavities, with the isatin scaffold in the substrate cavity and the C-5 styril replacement reaching into the entry cavity. The C-6 location of isatin, in contrast to C-5, points to the bottom of the substrate cavity. According to the crystal structure of human MAO-B, extending a C-6 styril side string into the entry cavity is only conceivable if the isatin scaffold uses an altered binding more than isatin. Analogues 7 and 7a were synthesized and subjected to evaluation of MAO inhibitory activity. From the biological evaluation, we can very clearly see that compounds 7 and 7a showed approximately 200- and 19-fold more potency toward MAO-B with IC_{50} values of 41.7 nM and 444 nM, respectively. Apart from the above study, they had also performed a computational study where the higher binding affinity of the (E)-styrylsatin derivatives in contrast to isatin is best clarified by the capacity of the styril isatins to bridge both the enzyme’s substrate cavity and entry cavity, according to molecular docking experiments with MAO-B. The analogues’ weaker binding to the hMAO-B Ile199 Ala mutant gives experimental support for this theory. In contrast to compound 35 (CSC) (Figure 9), changes in the comparative geometries of the aromatic rings for MAO-B and MAO-A explain the lower selectivity of (E)-styrylsatin derivatives between MAO-B and-A.

With an enzyme–inhibitor design of MAO inhibitors, it was shown that the endogenous chemically synthesized isatin is a moderately powerful inhibitor of human MAO-B. With the KI value of the 3 μM dissociation constant with a KI value of 15 μM, isatin also inhibits human MAO-A. According to the 3-D structure of a complex between hMAO-B and isatin, within the substrate cavity, isatin interacts with the C-2 carbonyl oxygen and dioxindolyl NH hydrogen linked to ordered water compounds in the active site. The entry cavity of MAO-B is left empty by this binding method. Isatin’s structure when linked to MAO-A has yet to be identified. Caffeine, a tiny molecule with a KI value of 3.6 μM, is a mild inhibitor of MAO-B. Caffeine’s inhibitory efficacy is significantly boosted when the caffeinyl ring is substituted with a styril side string at C-8. Molecular docking experiments of compounds 7 and 7a in the active site of MAO-B were undertaken to give additional insights. The crystallographic structure of the complex between hMAO-B and the reversible inhibitor safinamide was chosen for this purpose (2VSZ). The docking computations were carried out with Discovery Studio 1.7’s Ligand Fit program.
following a previously published technique. The best docking solution was discovered. As a result, analogues of compounds 7 and 7a have been recognized as possible new probes for MAO-B and MAO-A binding sites. The outcomes suggest the concept that small compound inhibitors of these flavoenzymes could boost their inhibitory potencies by exchanging side strings that enable interaction to both the entry and substrate cavities of MAO-B. MAO-Ile-199 B’s “gate” side string appears to be significant in identifying reversible blockers, potentially through linking with the styryl side strings of molecules 7−7c, as well as other aspects that will need extra structural data to discover. In contrast to the results with compound 35, the startling discovery that (E)-styrlylisatins are also competitive inhibitors of MAO-A reveals that relative geometries are key considerations in MAO inhibitor design.53 Figure 11a concludes that the C-6 position on styryl substitution decreases MAO-B inhibition, and the C-5 position on halogen-substituted styryl increases MAO-B potency. The C-5 position on halogen styryl increases MAO inhibition, and C-6 substitution is more selective for MAO-B.

When paired with indirectly acting sympathomimetic amines like tyramine, which is found in some foods, MAO-A inhibition has the potential to cause cardiovascular consequences. The amount of tyramine that reaches the systemic circulation is reduced because tyramine is normally metabolized by MAO-A in the gut wall. An irreversible suppression of MAO-A impairs normal tyramine metabolism in the stomach, resulting in higher amounts of tyramine in the bloodstream.53 Reversible blockers, on the other hand, have no effect since the inhibitor is quickly replaced by tyramine, which is then properly metabolized by MAO-A. These findings indicate that selective MAO-A inhibitors or MAO-A/B combination inhibitors should interact with MAO-A in a reversible manner. An additional benefit of reversible inhibition is that if the medicine is removed from the tissues enzyme activity quickly returns. Because the turnover rate for MAO biosynthesis in the human brain can be as high as 40 days, recovery of enzyme function after discontinuing irreversible MAO inhibitor medication could take several weeks.54 As a result, various investigations are underway to find novel MAO inhibitors that attach to the enzymes in a reversible manner. Isatin is an illustration of a reversible MAO-A/B combination inhibitor. Isatin is an endogenous type of molecule which suppresses human MAO-A and -B. Its enzyme−inhibitor Ki values for the two enzymes are 15 and 3 M, respectively. Although the X-ray crystal structure of isatin in complex with MAO-A has been discovered, the 3D structure of isatin within the binding center of hMAO-B remains unknown. The MAO-B substrate cavity to isatin is bound, according to the structural model, with the C-2 carbonyl group of the dioxo indolyl scaffold oriented toward the FAD cofactor. Isatin’s NH and C-2 carbonyl oxygen are stabilized by hydrogen bonding to water molecules in the MAO-B substrate cavity. The fact that isatin discovered in the MAO-B substrate cavity leaves the enzyme’s entrance cavity empty is remarkable. C-6 and C-5 isatin compounds were previously investigated as possible hMAO-A and -B blockers based on this discovery and modeling studies. To gain a better understanding of the problem, small molecules of 7 and 7b in the active site of MAO-A were investigated using LigandFit, as previously reported. The crystallographic structure of human recombinant MAOA in association with the reversible inhibitor harmine was chosen for this purpose (2Z5X). The dioxoindolyl rings of 7 and 7b are docked near the FAD (Figure 11b), with the styryl side chains extending toward the active site cavity opening in the top-ranked docking solutions. These binding modes are analogous to the MAO-B binding modes. The dioxoindolyl ring of 7a, which is rotated 180° in comparison to that of 7, is also equivalent to the docking findings achieved using MAO-B.55

S-Sulfanylphthalimidines were discovered to be effective and selective MAO-B inhibitors by Van der Walt et al.56 The benzylsulfanyl side string is especially well suited for enhancing phthalimide’s MAO-B inhibiting activity in this way. Compound 37 (Figure 12) (IC50= 0.0045 M) has a 30,000 times higher potency than phthalimide (IC50= 134 M). This example demonstrates the significant of the C-5 side string for MAO-B inhibitory activity. According to this study, S-sulfanylphthalimidines are feasible lead molecules for the development of antiparkinsonian drugs due to their MAO-B inhibitory effectiveness and suitable selectivity profiles. It is worth mentioning that S-sulfanylphthalimidines with significant MAO-B inhibitory characteristics can be created with a number of C-5 substituents from a design standpoint. This

![Figure 11. (a) Structure−activity relationship for C-5 and C-6 styryl-based isatin derivatives. (b) Compound 7 with MAO-B interactions.](https://doi.org/10.1021/acsomega.2c01470)
means that modifying the C-5 side string to develop the compound’s properties is less likely to decrease MAO-B inhibition strength.56

In SAR, this study concludes the sulfanyl chain increases MAO-A as well as MAO-B potency (Figure 13). Halogen-substituted benzylsulfinyl increases MAO-B inhibition.

When coupled with dietary tyramine, MAO-A inhibitors might cause substantial side effects. When the MAO-A-catalyzed breakdown of tyramine in intestinal endothelium is blocked, excessive levels of tyramine reach the systemic circulation. Tyramine, an indirectly acting sympathomimetic amine, induces peripheral adrenergic neurons to produce noradrenaline, causing a severe hypertensive reaction that can be fatal.57 As a result, MAO-A inhibitors have had little therapeutic application. Reversible MAO-A inhibitors, such as moclobemide, were found recently and are regarded to be safer than irreversible MAO-A inhibitors. Moclobemide, for illustration, suppresses the tyramine reaction while being effective as an antidepressant. As a result, nonselective MAO blockers used to treat Parkinson’s disease should be reversible in the best-case scenario.55 Based on these concerns, Strydom et al.59 looked into the prospect of phthalide derivatives (Figure 14) acting as reversible MAO inhibitors. Phthalide shares structural similarities with isatin and phthalimide, two small compounds that have been demonstrated to be good scaffolds for developing highly potential MAO inhibitors. The benzoxyl moiety has been substituted for isatin at both the C-5 and C-6 locations, yielding strong MAO-B inhibition. When compared to the C-6 location, substitution on the C-5 site is more pleasing for effective MAO-A and -B inhibition. Similarly, substituting the benzoxyl moiety for the phthalimide at the C-5 location resulted in compounds with significant MAO inhibitory properties. It has been demonstrated that C-6-substituted phthalide analogues inhibit MAO-A and -B. Several of the analogues show significant inhibition of both MAO isoforms, indicating that they are dual MAO-A and -B inhibitors, despite the fact that the phthalides mostly inhibit MAO-B. Furthermore, the data suggest that phthalides have a reversible interaction with MAO-A and -B, despite the fact that the phthalides mostly inhibit MAO-B. Furthermore, the data suggest that phthalides have a reversible interaction with MAO-A and -B. Reversibility and dual MAO-A/B inhibition are two required properties for creating antiparkinsonian medications. It is worth mentioning that phthalides with effective MAO-A and -B inhibitory effects can be synthesized using a variety of C-6 substituents. This is beneficial for improving the characteristics of these compounds since changes to their structures, mainly the C-6 substituent, are less likely to reduce MAO inhibition. C6 amino
substituents, on the other hand, have no effect on MAO inhibition, unlike oxy substituents. According to the literature, 8-aminocaﬀeines are mild MAO inhibitors, but 8-oxycaﬀeines are relatively powerful MAO inhibitors (compounds mentioned). According to the findings, the phthalide derivatives (Figure 14) are now MAO-A inhibitors as well. In fact, the IC₅₀ values of 12 of the 19 analogues were in the submicromolar range (0.096–0.629 μM). With an IC₅₀ value of 0.0096 μM, 41 was the most effective MAO-A inhibitor. This drug is also an effective inhibitor of MAO-B (IC₅₀ = 0.0062 μM). 41 is 16 times more selective for MAO-B than MAO-A, according to the SI value. The remaining phthalide analogues examined, with the exclusion of 39, demonstrated selective inhibition of MAO-B (SI = 10–214). Despite the fact that the phthalides are MAO-B-specific antagonists, most homologues have substantial MAO-A inhibitory effects; therefore, they could be used in situations when both MAO-A and -B reduction is required. Even though all of the phthalide derivatives studied here are effective MAO-A and -B blockers, the following phthalides are highly powerful dual MAO-A/B inhibitors: 38, 41, 42, and 43. These drugs have IC₅₀ values for both MAO isoforms that are less than 0.2 μM. Compound 40, another powerful dual inhibitor, was discovered to be slightly selective of the phthalides tested (SI = 10). In this study we conclude that after substitution on the benzyloxy group, the CF₃ > I > Br > Cl > H order increases MAO-B potency (Figure 15). Increasing the benzyloxy chain did not affect the MAO-B inhibition very much but in the MAO-A case increased the affinity toward MAO-A.

Tripathi et al.⁶⁰ investigated the design, development, and in vitro assays of 3-hydroxy-3-phenacyloxindoles that originated from isatin and compared their MAO-B and -A inhibitory and binding prospects to those of reference compounds. The mechanisms of inhibition of the most potent inhibitors against MAO-A and -B were investigated using kinetic studies. The capability to inhibit MAO-A and -B was investigated in vitro using a number of isatin 3-hydroxy-3-phenacyloxindole derivatives (Figure 16) that were designed, synthesized, and examined. Several isatin derivatives have been recognized that block MAO-B and -A while causing little or no neurotoxicity. Isatin derivatives (isatin and (E)-5-styrylisatin), as well as a few reference MAO inhibitors, exhibit potent MAO inhibitory activity (clorgyline, harmine, selegline, and rasagline). Isatin analogues (isatin and (E)-5-styrylisatin), as well as a few reference MAO blockers, show potent MAO inhibitory effects. The synthesis, in silico and in vitro MAO-B and MAO-A inhibition studies of 3-hydroxy-3-phenacyl-oxindole molecules derived from isatin, as well as comparisons of their MAO-B and MAO-A binding and inhibition capacities to those of reference inhibitor molecules are all part of this research. The modalities of inhibition of the most effective inhibitor molecules 49 and 47 against MAO-A and MAO-B, respectively, were studied using kinetic testing. Furthermore, docking simulation results (Autodock 4.2) were used to calculate the free energy of binding (DG) and inhibitory activity constant (KI) values of the MAO-B and MAO-A inhibitors that were experimentally investigated as well as to gain structural insight into the inhibitor substances’ binding

![Figure 15. Structure–activity relationship for phthalide-based derivatives.](image)

![Figure 16. Structure of phenacyloxindole analogues](image)
modes and types of interactions inside the active sites of MAO-A and MAO-B. SAR analyses disclosed several structural characteristics significant for the strength and selectivity of the suggested derivatives. The first is a succession of hydroxylations, one of which should be isatin’s C3 hydroxylation combined with the 3-phenacyl ring’s para hydroxylation. In addition to the hydroxy group, bromination at the para position of the 3-phenacyl side chain is critical for MAO-A activity and selectivity. The analysis clearly shows that compound 44 has the highest activity, with a selectivity index of 60.44, followed by compound 47 and compound 51, which have SIs of 8.54 and 37.61, respectively. Apart from compounds 52, 58, 62, and 63, all compounds displayed \( \pi-\pi \) interactions; \( \pi-\pi \) interactions with residue Tyr326 were identified in compounds 47 and 50, respectively, and with Tyr398 in compounds 53–56, 51, and 59–61. Furthermore, compounds 46 and 48 exhibit \( \pi-\pi \) interactions with Phe343; similarly, compounds 53–56, 60, and 61 have \( \pi-\pi \) interactions with Tyr435. A \( \pi-\sigma \) interaction with Tyr435 stabilizes compounds 49 and 60 as well as compounds 54 and 59 with FAD and molecule 59 with Ile199. Furthermore, hydrogen bond interactions with Tyr435 were identified for compounds 45, 49, 57, 58, and 60 as well as 45, 49, 57, 62, and 63 for the FAD cofactor. Figure 17 concludes that the halogenated phenyl ring on the R-1 position of isatin increases MAO-B as well as MAO-A potency. The benzyl moiety at the R-2 position of isatin increases affinity toward MAO-A.

Tavari et al. discovered that isatin is a relatively effective MAO-B- and MAO-A inhibitor, with IC\(_{50}\) values of 12.07 μM and 22.54 μM, respectively, which are consistent with prior findings. All of the novel test molecules effectively inhibited MAO-A (IC\(_{50}\): 4.31–22.75 μM). When the fluorophenylsulfonyl moiety (Figure 18a) was added to the 5 positions of isatin, the molecules retained MAO-A inhibitory action (IC\(_{50}\) = 21.73 μM and 22.75 μM) and were even better MAO-A
inhibitors (65, IC_{50} = 8.26 μM) than isatin. The MAO-A inhibitory activity of molecules with a propargylamine scaffold on the N-position of the isatin scaffold was two times higher.

The fluorophenyl amine moiety had a key role in the multipurpose activity of the produced compounds, as evidenced by molecules 64 and 65. They are promising contestants for antiapoptotic research, lead chemical development, and multifunctional drug design since they have good inhibitory activity against MAO-A and MAO-B, as well as great inhibitory activity against caspase-3. Compounds 66, 64, 65, and 67 (Figure 18), MAO-A-selective molecules with weak MAO-B and caspase-3 inhibitory bioactivity, show that adding the propargylamine scaffold only increased MAO-A inhibitory activity. Despite the lack of caspase-3 activity, the groups in these molecules may work in a totally diverse way compared to what was explored in this investigation. As a result, these compounds show significant promise as novel multifunctional neuroprotective drugs for the medication of Alzheimer’s disease, Huntington’s disease, and Parkinson’s disease, while additional research is needed to fully assess their potential in neurodegenerative disease treatment. The binding mechanisms of compound 65 and its propargylamine homologue 67 in MAO-A and -B were investigated, utilizing molecular docking to gain more understanding. The Brookhaven Protein Data Bank (www.rcsb.org/pdb) was used to get the structures of human MAO-A cocrystallized with harmine (PDB entry: 2Z5X) and human MAO-B cocrystallized with safinamide (PDB entry: 2V5Z). The isatin moiety binds in the polar area of the substrate cavity in the vicinity of the FAD cofactor and the "aromatic sandwich" formed by Tyr398 and Tyr435 in the best-ranked docking solution of compound 65 inside the active site of MAO-B (Figure 18b). This dioxoindolyl ring binding orientation is identical to that of isatin cocrystallized inside the active site of recombinant human MAO-B. Ile199 is located in the enzyme’s entry cavity, and the C-5 fluorophenylsulfonyl side chain of 65 extends beyond it. The cocrystallized inhibitor safinamide, which covers both active site cavities, has a similar binding orientation. In contrast to its MAO-B binding orientation, the isatin ring is located in the region of the FAD cofactor in the best-ranked docking solution of 67, as

![Figure 19. Structure–activity relationship for phenylsulfonyl-based isatin derivatives.](image)

![Figure 20. Structure of piperonylic acid based isatin derivatives.](image)
shown in compound 65. Although the propargylamine moiety is accommodated in the MAO-A active site cavity, its orientation prevents interaction with the FAD cofactor. The isatin moiety’s binding in the polar area of the substrate cavity in both 65 and 67 and probable interactions with adjacent amino acid residues may be the explanation for their MAO-A inhibitory effect. As observed in MAO-B modeling experiments, the para-fluorophenylsulfonyl moiety also exhibited a better orientation and lower binding energy when compared to its meta and ortho counterparts.61

In Figure 19, SAR concludes that the R-position of isatin propargyl increases MAO-A selectivity. The C-5 position at phenylsulfonyl in the position of halogen also affects the MAO affinity. Vishnu et al.62 synthesized a few isatin-based derivatives of piperonylic-acid-derived hydrazones (Figure 20). In a study, they wanted to develop new multitarget-directed ligands that could inhibit both MAO and ChE. The researchers then used the MTDL method to develop and synthesize a variety of piperonylic-acid-derived hydrazones with an isatin pharmacophore that had a variety of steric and electronic properties, and they used molecular docking and enzyme screening assays to estimate their pharmacological properties (in vitro ChE and MAO inhibition assays). Based on early in vitro MAO and ChE inhibition investigations, we discovered two lead compounds, molecules 68 and 69, that exhibit dual inhibitory activity against both MAO and ChE. The majority of the molecules was found to be more selective for MAO-B than MAO-A, with 69 being more than 50 times more active for MAO-B over MAO-A.62

The ortho- and p-dichlorobenzyl group at the R-1 position of the isatin ring has an MAO-A activity that is ten times that of the p-fluorobenzyl analogue, 72, 71, and so on. Compound 72 (Figure 19) which includes a p-fluorobenzyl group at the R-1 location has significantly lower activity than the other benzyl-substituted analogues. Molecules 69 and 65 with aliphatic propargyl and allyl substituents at the R-1 location were more active than benzyl-substituted analogues, with the exception of 73, which showed similar results to 69. In compounds 72 and 73, substitution of p-fluoro or dichlorobenzyl groups at the R-1 location increased MAO-B inhibitory action compared to the unsubstituted benzyl derivative 71. Molecules 72 and 73 were three times more selective for MAO-B than MAO-A, whereas 71 was the least selective of the molecules examined. With the exception of the p-fluorobenzyl derivative 72, which displayed activity similar to the allyl analogues 70, molecules 69 and 70 with aliphatic propargyl and allyl substituents at the R-1 site were more active than benzyl-substituted analogues. Although it was more selective and active than 71, adding a chloro group to the R-2 position 68 did not result in a significant increase in activity.62 Overall, this study concludes that aliphatic propargyl and allyl substituents are more active than benzyl for MAO inhibitory activity (Figure 21). Substitution of halogens and electronegative groups does not affect MAO potency very much.

Many MAO-B inhibitors, including irreversible types like selegiline and rasagiline, have been produced for use in both Parkinsons and Alzheimers treatment. The propargylamine moiety is a frequent kind of irreversible inhibitor that covalently binds to the FAD cofactor and can produce major adverse effects such as tyramine-induced hypertension. To address this issue, a new family of inhibitors were created that work by inhibiting MAO-B in a reversible manner. Safinamide, which is used to treat Alzheimer’s disease, is now the only FDA-approved reversible MAO-B inhibitor. Furthermore, isatin, an endogenous small molecule, is reported as a reversible inhibitor of an MAO enzyme that forms hydrogen bonds with conserved water molecules in the substrate cavity near the FAD cofactor.62 Some isatin-based drugs have been demonstrated to suppress MAO-B activity in this regard. They used a docking approach to compare the interactions of freshly synthesized compounds with reference MAO-B inhibitors over the MAO-B active site. Based on the IFD score, the top-scoring pose of all compounds was chosen and examined for analysis and free binding affinity computation with the help of the MM-GBSA method. All of the test inhibitors bind to the active site of the MAO-B cavity. All of the inhibitors bind to both the entrance cavity, which is surrounded by residues Leu171, Ile199, and Tyr326, and the substrate cavity, which is surrounded by residues Tyr60, Ile168, Cys172, Gln206, Phe343, Tyr398, and Tyr435; this is similar to the position taken by the reference MAO bionhibitor, safinamide. Safinamide’s amide side chain was pointed toward the FAD molecule and the fluorobenzyl group. Hydrogen-bonding
interactions with the inhibitors were found at residues Tyr188, Ile199, Tyr326, Ile198, Gln206, Tyr435, and Cys172. Compound 68 hydrogen bonded with residues Ile199, Tyr326, Gln206, and Ile198, whereas compound 69 hydrogen bonded with residues Ile199, Tyr326, Gln206, and Ile198. Compound 70 formed hydrogen bonds with Gln206, and 70 reacted with Ile198. Gln206, Cys172, and Ile198 were hydrogen bonded with a molecule 71. Compound 72 has comparable hydrogen-bonding affinities with Gln206 and Ile198 (Figure 21). Based on these findings, it was determined that p-alkyl and hydrogen-bonding interactions are responsible for the majority of the powerful drug inhibition of MAO-B.

Ahmadi et al. created 3-imino indolin-2-one compounds (Figure 22a) and tested them biologically for their inhibitory profiles of MAOs. Recently, the synthesis of 3-imino indolin-2-one compounds was performed using reflux circumstances with a wide range of solvents. All of the produced compounds showed greater free binding affinity than isatin, with the exception of molecule 3-imino indolin-2-one (known as a reversible noncovalent MAO-B inhibitor). Compounds 74, 75, 77, 76, and 78 exhibit greater free binding energy than selegiline when compared to the values shown in the MM-GBSA column. This study suggested that isatin analogues had a high affinity for the MAO-B enzyme’s active region.

The molecule isatin, which has a molar mass of 147.13, is a reversible inhibitor of hMAO-B with a modest affinity for this specific target. In our test, all molecules showed good affinity toward both the MAO enzymes. Here the compounds were subjected to Induced-Fit docking, and it was observed that isatin interacts weakly with Ile198, Leu171, and Ile199 and creates a bridging H connection with the FAD molecule via a water molecule bond. However, the isatin analogues showed affinity greater than that of isatin. Among these synthesized compounds, 76 showed the highest free binding energy and was seen to have an extended conformation to the entrance cavity of the substrate from the flavin ring location. The imino group of 76 was sustained through the MAO-B active center by creating a hydrogen bond interaction with Tyr435 in the same way as the selegiline tertiary amine was sustained, except for the longer MD simulation period. Interestingly, within the first 12 ns of MD simulation time, compound 76 showed an interaction with Lue171 and Cys172 (Figure 22b) located at the center of the substrate cavity via hydrophobic binding. The Ile199 side chain was seen along the boundary of the active center, which correlates to the open conformation, in the enzyme complex with compound 76, whereas the stated residue side chain moved forward toward the catalytically active cavity in the selegiline-bound state. In conclusion, from this literature, we learn that these 3-imino indolin-2-one compounds maybe good inhibitors, provided they showed good drug profiling based on the in silico analysis as well as the MTT assay.
Fragment-based drug discovery (FBDD) involves discovering tiny chemical fragments that may only interact weakly with the receptor and then develop or integrate them to create a lead with a greater affinity. Based on the crystallographic structure of hMAO-B isatin, it was found that isatin was bound in the substrate cavity, and a different compound 36 was bound to the entrance cavity. From this they supposed that to grow that particular fragment in order to develop compounds that can occupy the region in the entrance cavity, an increased selectivity and higher potency against MAO-B has to take place. It was seen that among both the carbonyl groups one is selectivity and higher potency against MAO-B has to take place. It was seen that among both the carbonyl groups one is seen in the hydrophobic environment. Thus, they came to a conclusion that by modifying the particular carbonyl groups they can produce higher binding affinity compounds.

Surprisingly, they were not able to see many good results, as expected by the modification of carbonyl groups. The PDB ID used by them is 2XFP which contained the isatin and compound 36 complex. A comparison study with PDB ID 2V5Z, a complex which contained safinamide as its cocrystal ligand, focused on five different scaffolds (Figure 23) and their derivatives (Figure 24). A wide range of derivatives of all

![Structure of benzyloxy-based isatin derivatives.](image)

the five scaffolds were synthesized and subjected to their MAO inhibitory activity by Cheng et al. Scafold A was created by the extension of an aromatic moiety from the fifth position of isatin, which has a strong affinity for hMAO-B, and Scaffold B with an indolin-2-one frame was created by cleavage of the 3-one group off the isatin core of Scaffold A. Nine molecules were then produced (Scaffold D). The sp2-hybridized carbon in the 3-one of isatin was altered to sp3 in this scaffold, resulting in the CH3 groups being linked to this sp3 hybrid carbon and oriented differently from the 3-one of isatin. 1,3-Dihydro-2H-benzo-imidazol-2-one is the frame of Scaffold E. Scaffold E was created by converting the isatin portion of Scaffold A’s 3-one group to a tertiary amine. As a result, none of the synthesized compounds showed good inhibitory activity. Compound 76 was created by extending a (3,4-dichlorobenzyl) oxy moiety from isatin’s 5 location, and it possesses outstanding potency (IC50 = 0.003 μM) and isozyme selectivity (SI = 38.933) against hMAO-B, which is followed by 79, 80, and 82 with IC50 values of 0.046 ± 0.007 μM, 0.073 ± 0.004 μM, and 0.102 ± 0.023 μM, respectively. While comparing the best four compounds of the A series, it is clearly seen that the compounds exhibit activity in the order 81 > 79 > 80. Compound 83, a poor hMAO-B inhibitor with an IC50 value of 7.68 M, was created by changing the 3-one to a nonpolar methyl group, which has a similar size but a diverse charge distribution.

The IC 50 value of 83 toward hMAO-B is just 7.68 μM, compared to 2560 times that for 81 (IC50 = 0.003 μM). Among the 26 compounds synthesized of the E series, only 8 compounds showed activity toward the hMAO-B enzyme, and from the 8 compounds that showed hMAO-B inhibitory activity, compound 83 showed the maximum hMAO-B inhibitory activity with an IC50 value of 7.68 ± 0.58 μM followed by 86, 84, and 85 with IC50 values of 34.30 ± 0.97 μM, 56.40 ± 1.12 μM, and 59.63 ± 1.21 μM, respectively. Overall, this SAR (Figure 25) study concludes that, by increasing the length of the benzyloxy bridge, a decrease in the MAO inhibition occurs. The benzyol moiety when substituted with halogen also affects MAO-B inhibition. Isatin in the 3-one group when replaced with an amine group may cause no inhibition or may affect MAO inhibition in an uncontrollable manner.

### CONCLUSIONS AND FUTURE PERSPECTIVE

Isatin’s wide range of reactivity has made it a desirable building block for the creation of a variety of heterocyclic scaffolds. Isatin and their analogues, i.e., phthalide and phthalimide, are promising scaffolds biologically and medicinally, fulfilling the huge range of biological profiles. In conclusion, the benzyloxy and benzal moiety were substituted for isatin at both the
positions C-6 and C-5, resulting in strong MAO inhibition. A hydroxyl group substituted for isatin at the C-5 position strongly and selectivity inhibits MAO-A. Benzyl substitution on the N-1 location of isatin is more selective for MAO-A than MAO-B. Halogen group substitution also exhibits increased MAO inhibitory activity. Benzylxoy and benzylsulfinyl with a halogen substituent at the C-5 position of the pthlalimide increase the MAO efficacy. In the case of pthlalide, at the C-5 position, benzylxoy enhances the MAO-B as well as MAO-A inhibition. This overall SAR analysis study will be useful for researchers who work in MAO-related diseases. For the future perspective point of view, we can modify and chop the isatin C-3 keto group and introduce a natural hydrophobic moiety for better interaction, as the already discussed MAO pocket is hydrophobic and enclosed with aromatic and aliphatic residues. All this information will be helpful in the design and development of a new class of drugs for the therapy of neurodegenerative diseases.

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**Notes**

The authors declare no competing financial interest.

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