Melatonin and related compounds as antioxidants

Alexia Barbarossa, Antonio Carrieri, Alessia Carocci*

Department of Pharmacy-Drug Sciences, University of Bari "Aldo Moro", 70126 Bari, Italy

* *Corresponding author: e-mail address: alessia.carocci@uniba.it*

Abstract

Several pieces of evidence suggest that oxidative stress is involved in the onset and development of many diseases, such as neurodegenerative and cardiovascular disorders, some types of cancer, and diabetes. Therefore, finding strategies to detoxify free radicals is an active area of research. One of these strategies is the use of natural or synthetic antioxidants. In this context melatonin (MLT) has been proven to possess most of the required characteristics of an efficient antioxidant. In addition, its protection against oxidative stress continues after being metabolized, since its metabolites also exhibit antioxidant capacity. Based on the appealing properties of MLT and its metabolites, various synthetic analogues have been developed to obtain compounds with higher activity and lower side effects. This review addresses recent studies with MLT and related compounds as potential antioxidants.

Keywords: Melatonin, circadian rhythms, indole nucleus, antioxidants, oxidative stress, radical scavengers, neurodegenerative disorders.

1. INTRODUCTION

Melatonin (*N*-acetyl-5-methoxytryptamine, MLT, Figure 1) is a tryptophan derivative primarily produced in the pineal gland, during the dark phase of a 24 h light/dark cycle where it is released into the blood and cerebrospinal fluid [1]. Extra pineal sources of MLT were reported in the retina, bone marrow cells, platelets, skin, lymphocytes, Harderian gland, cerebellum, and gastrointestinal tract [2]. Suprachiasmatic nucleus (SCN) of the hypothalamus is the biological clock that regulates MLT synthesis and secretion over 24 h. A neural output signal, generated by the SCN, induces the synthesis of MLT at night by the pineal gland. The hormone is released into the third ventricle and subsequently the circulation. Light, in addition to tuning the SCN, acts to inhibit MLT synthesis. Because MLT is rapidly metabolized, plasma MLT levels are low during the day and high during the night [3]. High levels of MLT at night induce target organs to enter appropriate homeostatic metabolic rhythms which prevent the body from developing different disorders [4]. Interestingly, MLT levels are reduced with aging and the decrease of its levels is now considered as a risk factor for neurodegenerative diseases [5].

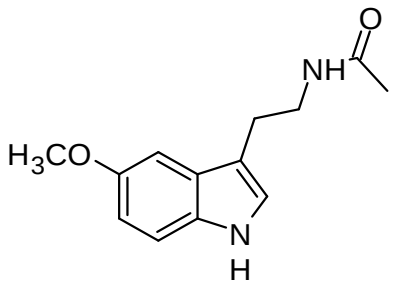


Figure 1: Melatonin structure.

Although MLT is best known to be involved in the circadian regulation of physiological and neuroendocrine function [6], the full spectrum of functional activities of this hormone includes antitumor [7], immunomodulatory [8], anti-inflammatory [9], pain modulator [10], neuroprotective [11], and antioxidant properties [12]. Moreover, many research findings provide scientific evidence for the protective role of MLT in a number of oxidative stress-related diseases, such as Alzheimer's

[13] and Parkinson's disease [14,15]. MLT exerts many of its pleiotropic functions through activation of high affinity G-protein coupled receptors MT_1 and MT_2, located at the plasma membrane [16] and possibly in mitochondria [17], that are differentially expressed and implicated in diverse biological functions and pathologies. A third MLT receptor with a lower affinity profile, found in the literature under the name of "MT_3", was identified as the cytosolic enzyme quinone reductase 2 [18]. Further MLT targets include the newly reported vitamin D receptor [19], calmodulin and other proteins that may act as MLT sensors [20].

Since 2019, MLT receptors have been enrolled in the unit of three-dimensional solved G protein-coupled receptors (GPCRs) and, besides the knowledge on the canonical 7TM topology, they have served fresh insights into the binding mode, selectivity, and mechanism of action of molecular entities that are recognize as MLT receptors ligands. Indeed, both agonists and antagonists bound data, as well as some mutants or G-protein (Gi) bound wild type receptor structures have been obtained see Table 1.

Table 1 MT_1 and MT_2 receptors deposited entries in the Protein Data Base (PDB)

	PDB code	Method	Res (Å)	bound ligand	G-protein	Mutant
MT_1	6ME2 [21]	XFEL[(a)]	2.80	ramelteon		
	6ME3 [21]	XFEL	2.90	2-phenylmelatonin		
	6ME4 [21]	XFEL	3.20	2-iodomelatonin		
	6ME5 [21]	XFEL	3.20	agomelatin		
	6PS8 [22]	XFEL	3.30	2-phenylmelatonin		
	7DB6 [23]	EM[(b)]	3.30	ramelteon	Gi	
	7VGY [24]	EM	3.10	2-iodomelatonin	Gi	
	7VGZ [25]	EM	3.30	ramelteon	Gi	
MT_2	6ME6 [25]	XFEL	2.80	2-phenylmelatonin		

	6ME7 [25]	XFEL	3.20	2-phenylmelatonin		H208A
	6ME8 [25]	XFEL	3.10	2-phenylmelatonin		N86D
	6ME9 [25]	XFEL	3.30	ramelteon		
	7VH0 [24]	EM	3.46	ramelteon	Gi	

Although these class of GPCRs lacks a clear and strong critical hot spot, such as the negatively charged aspartate of TM3 making salt bridge with the protonated nitrogen in the aminergic receptors, these achievements shed light on the ligand's molecular recognition process in MLT receptors, exerted through some key residues and critical interactions hereafter described. Both polar and aromatic interactions are mandatory in these receptors binding since the X-ray structures highlighted that Phe179, Asn162 and Gln181 in MT_1, and Asn174, Phe192 and Gln194 in MT_2, assist both endogenous and exogenous ligands by means of π–π aromatic stacking as well as properly oriented hydrogen bonds. In Figure 2, the critical residues of MT_1 and MT_2 receptors involved in the interaction with the main reference compounds of the melatonergic domain (2-phenylmelatonin, agomelatine and ramelteon) have been reported. However, regardless this evidence the most important structural motif is the presence of an entry channel locate at the lateral and apical moiety of the TM scaffold serving as ligand access to the binding site. As suggested by Stauch et al. [21] this feature might represent a diverse, probably allosteric, site that can be exploited in rational structure-based drug design and virtual screening campaign. Very interestingly these same channels might also look over the MT_1/MT_2 selectivity indeed the data published by Johansson et al. [25], evidenced in the solvent exposed part of the receptor this narrow opening. And this is very much indeed true also due to the very high sequence identity/homology in the MT receptors as evidenced in Figure 3.

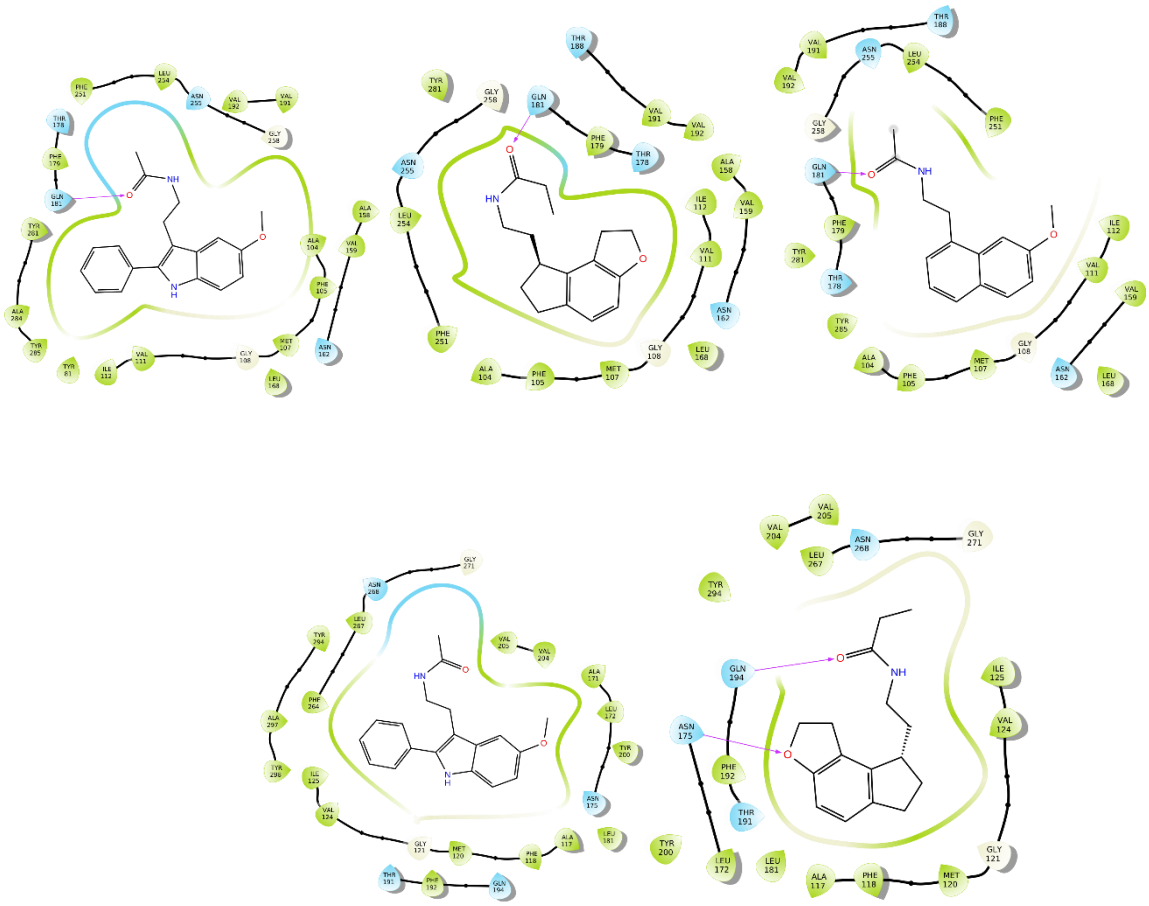


Figure 2: Critical residues of MT_1 (from left to right 6ME3 6ME2 and 6ME5) and MT_2 (from left to right 6ME6 and 6ME9) upper and lower respectively.

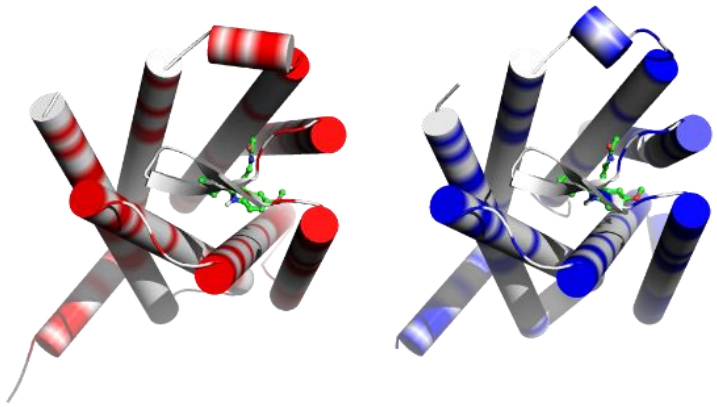


Figure 3: Observed mutations in the MLT receptors structures. Identical residues are reported in white, while non conserved positions of MT_1 (right) and MT_2 (left) are depicted in red and blue respectively.

Overall, crystallography studies applied on MT_1 and MT_2 furnished insights into the characteristics of these receptors as molecular targets in diverse process, including antioxidant activity endowed ligands.

MLT is a well-known antioxidant effective in reducing oxidative stress under a remarkably large number of circumstances. MLT has been shown as a specific antioxidant because of its amphiphilic feature that allows it to cross physiological barriers, thereby reducing oxidative damage in both lipid and aqueous cell environments. This property confers to MLT an advantage when compared to other antioxidants whose action is limited because of their solubility which affects their partitioning between intra- and extra- cellular compartments. MLT achieves its antioxidant action via direct and indirect mechanisms. It is a powerful free radical scavenger able to remove reactive oxygen (ROS) and nitrogen species (NOS). As an electron rich molecule, it interacts with free radicals through consecutive reactions giving rise to a free radical scavengers cascade, since its metabolites, like *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK) and *N*1-acetyl-5-methoxykynuramine (AMK) are also efficient free radicals scavengers even more potent than MLT itself [26,27]. In addition, MLT confers indirect protection against free radicals by stimulating the transcription and activity of antioxidant enzymes like superoxide dismutase and glutathione peroxidase, while suppressing the activity of pro-oxidant enzymes [28]. MLT regulation of antioxidant enzymes is supposed to be receptor-mediated. MLT has also been reported to chelate transition metals, which are involved in the Fenton/Haber–Weiss reactions, thus reducing the formation of the highly toxic hydroxyl radical [29]. Besides that, MLT protects lipids, protein, and DNA from oxidative damage, being highly concentrated in the mitochondria. There is ample evidence that MLT should be classified as a mitochondria-targeted antioxidant. It accumulates in mitochondria with high concentration, against a gradient, by an active transportation via mitochondrial MLT transporters. MLT protects mitochondria by scavenging ROS, inhibiting the mitochondrial permeability transition pore (MPTP), and activating uncoupling proteins (UCPs). Thus, MLT maintains the optimal mitochondrial membrane potential and preserves mitochondrial functions [30]. Despite the therapeutic potential of MLT in a wide variety of clinical conditions, the use of MLT in therapy could be complicated by the pharmacokinetic behaviour of the molecule, which is subjected to a considerable first-pass effect and to a limited bioavailability and plasma half-life after oral administration [31,32]. Thus, the development of new

compounds sharing the beneficial properties of MLT but presenting different pharmacokinetic profiles represents an interesting topic for researchers. During the past two decades, a great number of structurally different MLT receptor ligands have been reported in the literature [33]. Several melatonin-related compounds have been under investigation as antioxidant agents, most of them being indole-based compounds and showing a better antioxidant behaviour than MLT. These compounds could represent useful pharmacological tools to treat oxidative stress related diseases. This review aims to collect the updated literature data concerning MLT analogues and derivatives as potential antioxidant agents. A brief overview of the antioxidant MLT properties has been also reported.

1. Antioxidant activity of melatonin

Oxidative stress is a common feature of various pathological conditions, including metabolic, degenerative, and cardiovascular disorders, and cancer. In addition to enzymatic defence mechanisms, antioxidant compounds offer chemical protection against oxidative events. Among natural antioxidants, MLT is one of the most comprehensively studied. MLT has been shown to act as a powerful antioxidant, more effective than vitamin E, which plays a protective role both intracellularly and extracellularly [34]. Melatonin counteracts free radicals both directly and indirectly. The direct mechanism involves the buffering capacity of its aromatic indole ring reacting with reactive oxygen species (ROS) and reactive nitrogen species (RNS). As a result, some metabolites will be formed that display antioxidant properties via a cascade reaction mechanism, with consequent amplified effects [35]. An example is provided by the hydroxylation of MLT on C3 which forms the cyclic 3-hydroxymelatonin (C3-OHM), through a reaction that neutralizes the hydroxyl radical. C3-OHM is subsequently excreted in the urine, thus representing an ongoing scavenging index [36]. MLT-mediated neutralization of hydrogen peroxide and singlet oxygen leads to the

metabolite N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK). Its activities include the buffering of radical species, hampering DNA and protein damage and lipid peroxidation, and the decreased rate of death of cells exposed to hydrogen peroxide. The cleavage of AFMK pyrrolic ring brings to the N1-acetyl-5-methoxykynuramine (AMK) which inhibits NOS *in vitro* in a dose-dependent manner. AMK is a more powerful ROS scavenger when compared to AFMK. Besides, AMK scavenges NO generating a stable nitrosation product, modulates mitochondrial metabolism, and interacts with aromatic rings by forming adducts with tyrosine and tryptophan residues that lead to protein modification. Instead, when AMK neutralizes RNS, N1-acetyl-5-methoxy-3-nitrokynuramine (AMNK) and 3-acetoamidomethyl-6-methoxycinnolinone (AMMC) are formed [37]. Concerning the indirect antioxidant mechanism of action, it engages MLT receptors. Indeed, it has been shown that the activation of MT1 and MT_2 receptors by the ligand stimulates the expression and thus the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRd). MLT also protects antioxidant enzymes from oxidative damage and increases the synthesis of GSH and glucose-6phosphate dehydrogenase (G6PD), an enzyme fundamental for the first and rate-limiting step of the pentose phosphate pathway, in which NADPH is produced and then used for GSH recycling. It has been reported that MLT also increases antioxidant defences by epigenetically inducing Nrf2 and activating antioxidant response elements (AREs). AREs are located in the promoter region of genes coding for antioxidant enzymes thus, their activation leads to the transcription of many antioxidant proteins and enzymes that process reactive oxygen species and transport proteins [38,39]. Furthermore MLT has been shown to inhibit pro-oxidative enzymes as xanthine oxidase or oxidant generating genes such as inducible nuclear factor-kappa B, nitric oxide synthase, and cyclooxygenase-2 [40]. The measurement of the subcellular distribution of MLT has shown that the concentration of this indole in the mitochondria greatly exceeds that in the blood. Melatonin presumably enters mitochondria through oligopeptide transporters, PEPT1, and PEPT2. Thus, MLT is specifically targeted to the mitochondria where it seems to function as an apex antioxidant. In addition to being taken up from

the circulation, MLT may be produced in the mitochondria as well [41]. Since it has ready access to the intermembrane space and matrix of mitochondria where it highly concentrates, MLT seems to meet the criteria as a mitochondria-targeted, being as good as or better than the synthetic mitochondria-targeted antioxidants, MitoE and MitoQ (vitamin E and coenzyme Q10 coupled to the triphosphonium cation, respectively) in reducing oxidative damage and inflammation [42]. Besides the antioxidant protection, MLT plays critical roles in mitochondrial function such as regulation of respiratory chain complexes I and IV activities and protection of mitochondrial DNA from mutations and deletions [43]. It was recently demonstrated that MLT is synthetized in mice brain mitochondria and acts through the mitochondrial external membrane MLT receptor MT_1, preventing cytochrome c leakage and subsequent apoptosis, an action that has been defined by the authors as "automitocrine" to describe the unexpected intracellular organelle ligand–receptor pathway [17]. Several studies have demonstrated that MLT plays an effective role in preserving mitochondrial homeostasis, which may explain the protective effect of this molecule in mitochondrial dysfunction associate diseases as neurological and cardiovascular disorders [44]. The benefits of MLT have been investigated under a variety of pathological conditions associated with oxidative stress, as neurodegenerative disorders [45]. Parkinson disease (PD) is a chronic and neurodegenerative disease with motor and nonmotor symptoms. Increasing evidence documents that MLT has a profound influence on PD [15]. Multiple pathways are involved in the pathophysiology of PD, including apoptosis, mitochondrial dysfunction, and oxidative stress [46]. Several studies corroborated MLT effects on oxidative stress markers and mitochondrial complex 1 activity [43]. Preclinical and clinical studies have shown that MLT supplementation is an appropriate therapy for PD. Several studies corroborated the link between MLT administration and effects on multiple oxidative stress signalling in PD as superoxide dismutase (SOD) mitochondrial complex-I activity, and glutathione (GSH) [47]. Indeed, MLT, in a dosage between 10–30 mg/kg, increased SOD, mitochondrial complex-I activity, and GSH in substantia nigra (SN) in a rat model of PD induced by homocysteine. In addition, MLT reduced hydroxyl radical (·OH) and increased catalase in the SN of the same rat model of PD [48]. A recent clinical study

showed that the administration of MLT was associated with a dramatic decrease of lipoperoxides, nitric oxide metabolites, and carbonyl groups in plasma samples from PD patients whereas the catalase activity was improved with respect to the placebo group. Moreover, the melatonin-treated group exhibited a great increase in mitochondrial complex 1 activity and respiratory control ratio. Instead, the fluidity of the membranes was not affected [49]. A recent study revealed the protective roles of MLT in an *in vitro* PD model. Indeed, MLT was able to rescue cells from the toxic effects of 1-methyl-4-phenylpyridimium (MPP+) on dopaminergic cell death by inducing the expression of HSP70 (Heat shock proteins) that plays an essential role, as molecular chaperones, to prevent abnormal protein aggregation and misfolding and protect dopamine neurons from oxidative stress, inflammation, and apoptosis, all well-known pathological mechanisms of PD [50]. Alzheimer's disease (AD), one of the most common types of neurodegenerative diseases and the leading cause of dementia among the aged people, is characterized by progressive and chronic deterioration of cognitive functions. Oxidative stress, which is one of the earliest events in the pathogenesis of AD and may even precede the appearance of pathophysiological hallmarks of the disease such as senile plaques and neurofibrillary tangles, appears to be a major determinant of AD pathogenesis and progression. In fact, constant evidence of the injury mediated by ROS and reactive nitrogen species RNS is observed in most cellular macromolecules of AD brain. MLT seems to play a neuroprotective role as the potential treatment of AD [51]. Moreover, recent studies have shown that MLT could potentiate the proliferation and differentiation of neural stem cells in the hippocampus of adult mice [52]. Preclinical studies showed that MLT is able to restore cholinergic and glutamatergic neurotransmission in AD [53]. Moreover, clinical studies have shown that MLT is effective in patients with mild cognitive impairment (MCI), which is the prodromal stage of AD [54]. Thus, the development of novel melatonin-based therapies may be envisage as a potential treatment for patients with AD.

2. Melatonin analogues endowed with antioxidant activity

MLT features make this molecule a particularly appealing antioxidant, since they are in line with those described as required for an ideal antioxidant. Accordingly, the design and synthesis of MLT derivatives and analogues with improved antioxidant profile are an emerging research area Therefore, in the last years, several MLT synthetic analogues of MLT have been developed. The common strategy consists of modifying the existing groups or introducing new groups in the different sites of the indole ring, as well as replacing the indole nucleus with a bioisosteric one (Figure 4).

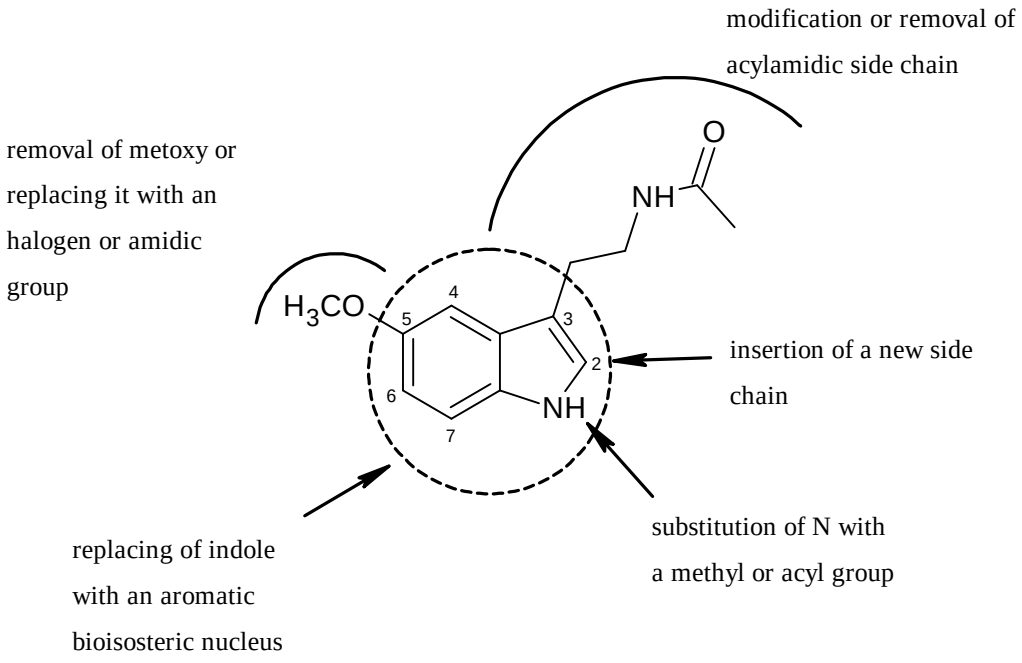
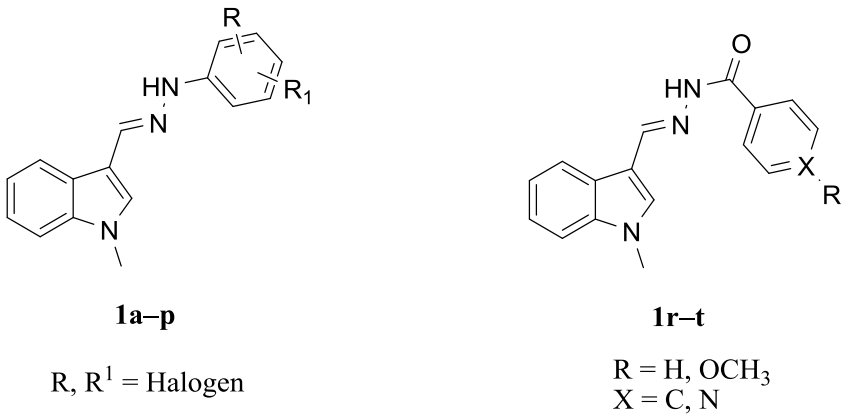


Figure 4. Main MLT structure modifications in synthetic analogues

In 2010, Shirinzadeh et al. [55] synthesized nineteen indole hydrazide/hydrazone derivatives (Figure 5) and investigated them *in vitro* for their potential antioxidant activity by three different assays: by evaluating their reducing effect against oxidation of a redox sensitive fluorescent probe, DCFH-DA, by investigating their protective effect against H_2O_2-induced membrane lipid peroxidation and by determining their inhibitory effect on 2,2'-azobis(2-amidinopropane hydrochloride) (AAPH)–induced hemolysis. Tests were performed on human erythrocytes chosen as a biological model since they are readily available cells sensitive to oxidative damage. In general, all the indole hydrazide derivatives were found to have potent antioxidant activity, even higher than MLT itself, with

differences in their relative potencies probably related to electronic distribution. Experimental results revealed that the lack of a methoxy group and the introduction of a methyl group at the nitrogen in the indole ring and the presence of a halogenated aromatic side chain resulted in much more active compounds than MLT itself, conceivably as an increased stability of the indole ring and delocalization of the electrons that help to scavenge free radicals by forming stable indolyl cation radicals. Compounds **1b** (R = 2-F, R^1 = H), **1c** (R = 2-F, R^1 = 4-F), **1m** (R = 3-Cl, R^1 = 5-Cl), **1k** (R = 2-Cl, R^1 = 5-Cl) and **1l** (R = 3-Cl, R^1 = 4-Cl) showed the best antioxidant activity. No significant antioxidant activity was observed in compounds **1r**, **1s** which have no halogen atoms in their structure.



1a–p

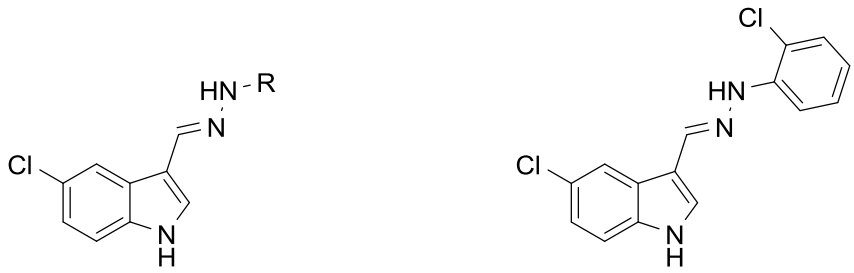
R, R^1 = Halogen

1r–t

R = H, OCH_3
X = C, N

Figure 5: Hydrazide/hydrazone derivatives structures (**1a–p,1r–t**).

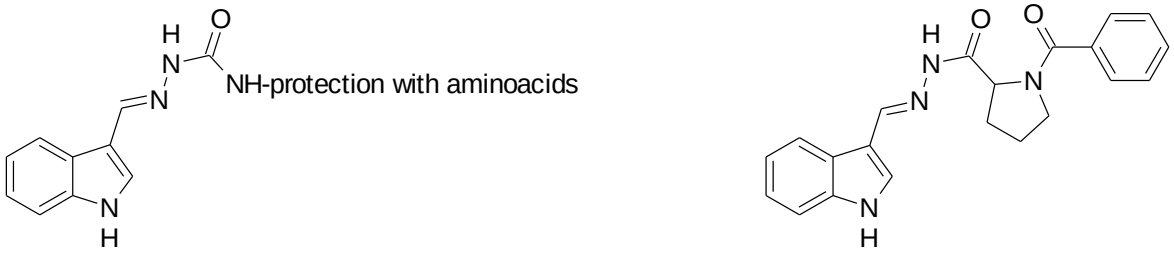
In 2012, Yılmaz et al. [56] prepared 5-chloroindole hydrazide derivatives (Figure 6) from 5-chloroindole-3-carboxaldehyde and phenyl hydrazine derivatives. Most of the compounds under investigation exhibited a substantial inhibitory effect on the superoxide radical scavenging assay at a concentration of 1 mM (79 to 95%). Moreover, these compounds were powerful scavengers of DPPH radical with notable IC_{50} values (2 to 60 μM). Particularly, compound **2** showed stronger inhibitory activity against MLT in the LP inhibitory assay at 0.1mM concentration (51%).



5-chloro indole derivatives **2**

Figure 6: 5-Chloroindole hydrazide derivatives structures.

In 2012, Suzen et al. [57] focused on the synthesis and antioxidant properties of 14 MLT-based analogues of indole amino acid and *N*-protected amino acid derivatives (Figure 7). All the tested compounds exhibited a similar scavenging capacity against DPPH radical with respect to MLT. However, among the investigated compounds, **3** proved to possess the best antioxidant activity, greater than MLT in the lipid peroxidation inhibition assay.

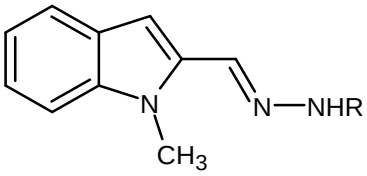


indol-aminoacid derivatives **3**

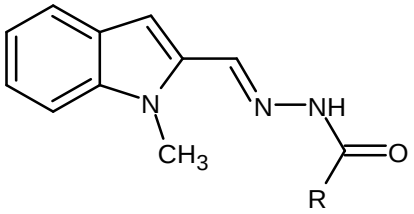
Figure 7: Indole amino acid derivatives structures.

In 2013, the same author et al. [58] prepared a series of indole based MLT analogue hydrazide/hydrazone derivatives (Figure 8) and investigated their protective effect against DCFH-DA oxidation in human erythrocytes. Furthermore, membrane stabilizing effect of all compounds was also investigated by lactate dehydrogenase leakage assay. In general, all the synthesized indole derivatives except **4e** and **4r**, were found to have potent antioxidant activity, even higher than MLT itself, differences in their relative potencies being probably related to electronic distribution. The authors observed that among mono-halogenated derivatives the presence of *o*- and *m*-halogenated

aromatic side chain increased the antioxidant activity (such as compounds **4g**, **4l**, and **4n**). Furthermore, the authors investigated the electrochemical behaviour of selective MLT by applying voltametric methods to get insight into their metabolism, owing to the oxidation mechanisms taking place at the electrode and in the body sharing similar principles. They observed that oxidation occurred firstly on the nitrogen atom of the indole ring leading finally to hydroxylation of the benzene ring.



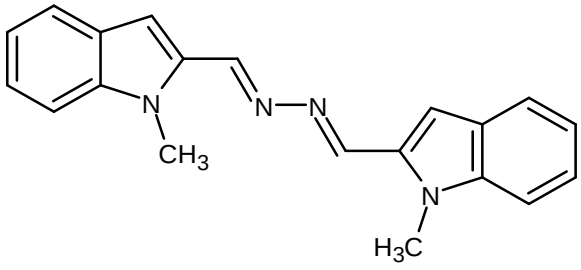
4a–s



4t,u

4a: R = H
4b: R = Ph
4c: R = 2-FPh
4d: R = 3-FPh
4e: R = 4-FPh
4f: R = 2,4-di FPh
4g: R = 2,5-diFPh
4h: R = 3,5-diFPh
4i: R = 2-ClPh
4j: R = 3-ClPh
4k: R = 4-ClPh

4l: R = 2,5-diClPh
4m: R = 3,4-diClPh
4n: R = 3,5-diClPh
4o: R = 2-BrPh
4p: R = 3-BrPh
4q R = 4-BrPh
4r: R = 2,3-diMePh
4s: R = 2,4-diMePh
4t: R = 4-Pyr
4u: R = 4-OMePh

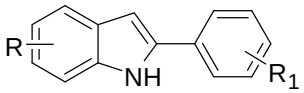


4w

Figure 8: Indole hydrazide/hydrazone derivatives structures (**4a**–**w**).

In 2013, Karaaslan et al. [59] synthesized a series of substituted 2-phenyl-1*H*-indoles structurally (Figure 9) related to the known indole-based antioxidant lead compound MLT, and the antitumour 2-

(4-aminophenyl) benzothiazole and 2-(3,4-dimethoxyphenyl) benzothiazole series. The research group assessed that 2-(4-aminophenyl)indoles (such as the 6-fluoro analogue **5b**) exhibited a considerable antioxidant capacity in the DPPH and superoxide radical scavenging assays (80% and 81% inhibition at 1 mM concentration of **5b**, respectively), analogously to MLT (98% and 75% at 1 mM). The lead compounds emerging with the most potent antioxidant activity were **5b**, **5h,** and **5i**. Overall, most of the substituted 2-(4-aminophenyl)-1*H*-indoles showed higher levels of antioxidant effect compared to their 2-(methoxyphenyl)indole counterparts.



5a–q

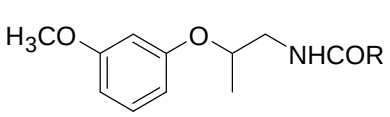
5a: R = 5-F, R_1 = 4-NH_2
5b: R = 6-F, R_1 = 4-NH_2
5c: R = 7-F, R_1 = 4-NH_2
5d: R = H, R_1 = 4-NH_2
5e: R = 5-Cl, R_1 = 4-NH_2
5f: R = 4-Cl, R_1 = 4-NH_2
5g: R = 6-Cl, R_1 = 4-NH_2
5h: R = 5-OMe, R_1 = 4-NH_2

5i: R = H, R_1 = 3,4-diOMe
5j: R = 5-F, R_1 = 3,4-diOMe
5k: R = H, R_1 = 4-OMe
5l: R = H, R_1 = 3,4,5-triOMe
5m: R = 5-F, R_1 = 3,4,5-triOMe
5n: R = 5-F, 7-Cl, R_1 = 4-OMe
5o: R = 5-F, 7-Cl, R_1 = 3,4-diOMe
5p: R = 5-F, R_1 = 3-F, 4-OMe
5q: R = 5-F, 7-Cl, R_1 = 4-NO_2

Figure 9: Substituted 2-phenyl-1*H*-indoles structures (**5a–q**).

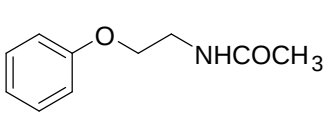
In 2013 Carocci et al. [60] tested some chiral *N*-(phenoxyalkyl) amides (Figure 10), selected among a series of potent MT_1 and MT_2 melatonergic ligands [61], for their antioxidant properties by measuring their reducing effect against oxidation of 2',7'-dichlorodihydrofluorescein (DCFH) in the DCFH diacetate (DCFH-DA) assay. Among the tested compounds, only *N*-[2-(3-methoxyphenoxy)propyl]butanamide (**6b**) displayed potent antioxidant activity resulting in slightly more active than MLT. This activity occurs in a stereoselective manner, since a significant difference between its enantiomers was observed; in particular, the (*R*)-enantiomer behaves as the eutomer. These results may allow the authors to rule out a possible interference of MT_1 and MT_2 receptors on

the antioxidant activity shown by **6b**, since both compounds **6b** and **6c**, which differ only for the acyl moiety are high affinity melatonergic agonists. The antioxidant activity of compound (*R*)-**6b** is not accompanied by radical scavenging ability, since these compounds, as well as MLT, showed only a weak DPPH inhibition activity pattern. Compounds **6b** and **6c** performed as Ca^{2+}/calmodulin-dependent kinase II inhibitor, too.



6a–c

6a: R = Me
6b: R = *n*-Pr
6c: R = c-Pr



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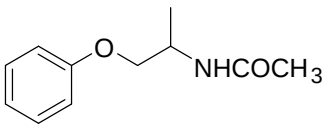

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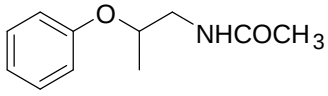

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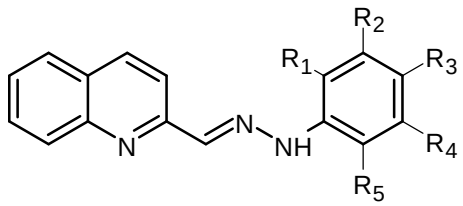
Figure 10: *N*-(phenoxyalkyl) amides structures (**6a–c,7–9**).

In 2016, Puskullu et al. [62] synthesized a series of quinoline-2-carbaldehyde hydrazone derivatives (Figure 11) as bioisosteric analogues of MLT. Results showed that all the quinoline derivatives possessed protective effects against ROS production on chinese hamster ovary (CHO-K1) cells, with an activity even higher than the parent compound MLT. Compound **10a**, the only with no halogen on the aromatic ring, demonstrated the best antioxidant activity. Particularly interesting were mono halogenated derivatives, which generated a better antioxidant effect in comparison with di-halogenated ones.



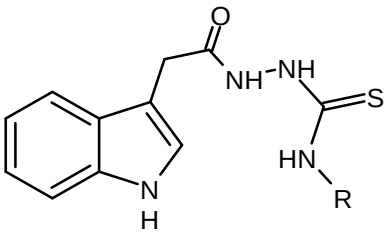
10a–q

10a: $R_1 = R_2 = R_3 = R_4 = R_5 = H$
10b: $R_1 = F$, $R_2 = R_3 = R_4 = R_5 = H$
10c: $R_2 = F$, $R_1 = R_3 = R_4 = R_5 = H$
10d: $R_3 = F$, $R_1 = R_2 = R_4 = R_5 = H$
10e: $R_1 = R_3 = F$, $R_2 = R_4 = R_5 = H$
10f: $R_1 = R_4 = F$, $R_2 = R_3 = R_5 = H$
10g: $R_2 = R_4 = F$, $R_1 = R_3 = R_5 = H$
10h: $R_1 = Cl$, $R_2 = R_3 = R_4 = R_5 = H$
10i: $R_2 = Cl$, $R_1 = R_3 = R_4 = R_5 = H$
10j: $R_3 = Cl$, $R_1 = R_2 = R_4 = R_5 = H$
10k: $R_1 = R_4 = Cl$, $R_2 = R_3 = R_5 = H$
10l: $R_2 = R_3 = Cl$, $R_1 = R_4 = R_5 = H$
10m: $R_2 = R_4 = Cl$, $R_1 = R_3 = R_5 = H$
10n: $R_1 = Br$, $R_2 = R_3 = R_4 = R_5 = H$
10o: $R_2 = Br$, $R_1 = R_3 = R_4 = R_5 = H$
10p: $R_3 = Br$, $R_1 = R_2 = R_4 = R_5 = H$
10q: $R_1 = R_3 = NO_2$, $R_2 = R_4 = R_5 = H$

Figure 11: Quinoline-2-carbaldehyde hydrazone derivatives structures (**10a–q**).

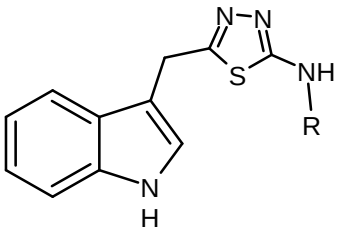
In 2016, the same research group [63] synthesized new indole based MLT analogues which possess triazole, thiadiazol and carbothioamides on the third position on the indole ring (Figure 12). *In vitro* antioxidant activity was investigated by evaluating their reducing effect against oxidation of the redox sensitive fluorescent probe DCFH-DA while their radical scavenging activity was assessed via the DPPH assay. Among compounds under study, several MLT analogues possessed antioxidant activity, whereas **11b** and **12e** were the most active of the series. Among newly synthesized compounds generally hydrazinecarbothioamide (**11a–h**) and 1,2,4-triazole-3- thiol derivatives (**13a–h**) were found to have high scavenging activity. Compound **11b** which was found to have the highest antioxidant effect in the cell based *in vitro* DCFH assay was found to have high scavenging activity in DPPH assay too. The authors suggest that the antioxidant effect of **11b** is probably a result of its radical scavenging activity. However, compound **12e** which was also found to have antioxidant activity according to the DCFH assay, was found to have very low scavenging activity in the DPPH assay, suggesting an alternative mechanism for its antioxidant activity. However, almost all hydrazincarbothioamide (**11a–h**) and 1,2,4-triazole-3-thiol derivatives (**13a–h**) were found to have

radical scavenging activity in the DPPH assay where no antioxidant activity was observed in the cell based DCFH assay. The authors supposed that this result could be due to limited availability of the compounds in cell cytosol because of the possible limited membrane passage, however, this assumption has not experimentally verified.



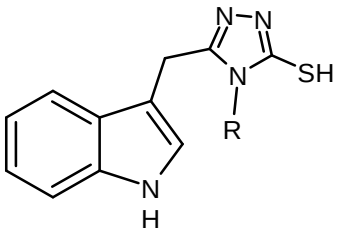
11a–h

11a: R = Et
11b: R = *n*-Pr
11c: R = CH_2Ph
11d: R = Ph
11e: R = 2,4-diFPh
11f: R = 3-FPh
11g: R = 2,4-diClPh
11h: R = 3-ClPh



12a–h

12a: R = Et
12b: R = *n*-Pr
12c: R = H
12d: R = Ph
12e: R = 2,4-diFPh
12f: R = 3-FPh
12g: R = 2,4-diClPh
12h: R = 3-ClPh



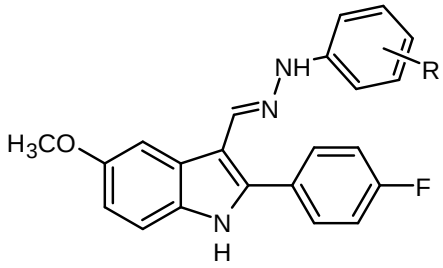
13a–h

13a: R = Et
13b: R = *n*-Pr
13c: R = CH_2Ph
13d: R = Ph
13e: R = 2,4-diFPh
13f: R = 3-FPh
13g: R = 2,4-diClPh
13h: R = 3-ClPh

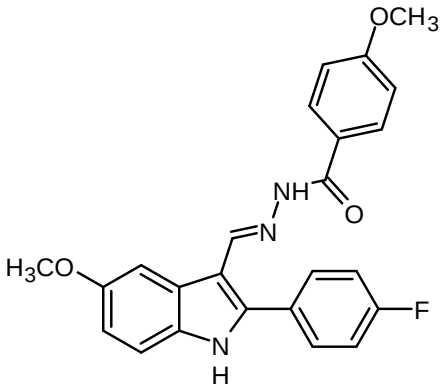
Figure 12: hydrazincarbothioamide (**11a–h**), 1,2,4-thiadiazole-2-amino (**12a–h**), 1,2,4-triazole-3-thiol (**13a–h**) derivatives structures.

In 2016, Gurer-Orhan et al. [64] synthesized a new series of indole-based hydrazide/hydrazone derivatives (Figure 13) and explored their antioxidant activity on ROS-induced DCFH-DA oxidation

in human erythrocytes. The possible inherent cytotoxicity of the compounds was investigated in CHO-K1 cells by lactate dehydrogenase leakage test. Protection of neuronal PC12 cells against amyloid β-induced damage was examined by MTT assay and their ability in reduction of ROS generation induced by amyloid β was tested. Most indole derivatives were found to have potent antioxidant activities, while they were devoid of inherent cytotoxicity against CHO-K1 and PC12 cells. Compounds **14a**, **14h**, **14j** and **14m** exhibited the highest activities among all compounds. MLT analogues bearing an *o*-halogenated aromatic moiety demonstrated successful antioxidant activity without inducing membrane-damaging effects. The authors found that compound **14o**, the only hydrazide analogue in the series and only compound with no halogen substitution, showed a slight prooxidant effect and did not show activity in any of the applied tests. They assessed that the new compounds have a great potential for designing new MLT-based indole derivatives as therapeutic agents for oxidative stress-related pathologies, especially Alzheimer's disease.



14a–n

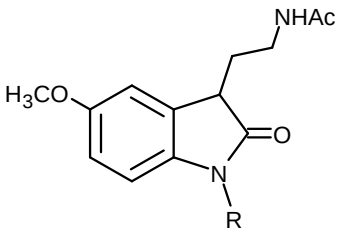


14o

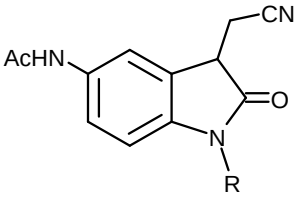
14a: R = 2-ClPh
14b: R = 3-ClPh
14c: R = 4-ClPh
14d: R = 2,5-diClPh
14e: R = 2,4-diClPh
14f: R = 3,4-diClPh
14g: R = 3,5-diClPh
14h: R = 2-BrPh
14i: R = 3-BrPh
14j: R = 2-FPh
14k: R = 3-FPh
14l: R = 4-FPh
14m: R = 3,5-diFPh
14n: R = 2,4-diFPh

Figure 13: Indole-based hydrazide/hydrazone derivatives structures (**14a–o**).

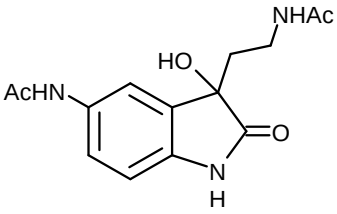
In 2017, Chesnokova et al. [65] prepared a series of 2-oxindole derivatives (Figure 14) which can bind MT_2 and MT_3 receptors. Data revealed that these analogues reduced intraocular pressure (IOP) in normotensive rabbits. Although maximal values of IOP reduction induced by these compounds did not exceed the one of MLT, the hypotensive effect of some analogues lasted for a longer period than that of MLT as for compound **15** which displayed the best hypotensive effect among the tested compounds. Antioxidant activity of the compounds under study was evaluated by changes in parameters of chemiluminescence kinetics in hemoglobin−H_2O_2−luminol model system. Test revealed that they possessed elevated antioxidant activity against hydroxyl radical and superoxide anion-radical even though there was no direct relationship between antioxidant activity of the tested derivatives and their ability to reduce IOP (as in the case of compounds ligands **21,22** which had low hypotensive activity (if any) but very high antioxidant activity. Most of derivatives exceed MLT in antioxidant activity thus the authors proposed them as promising candidates for the use in treatment of eye diseases accompanied by oxidative stress.



15: R = Me
16: R = H



17: R = H
18: R = Me



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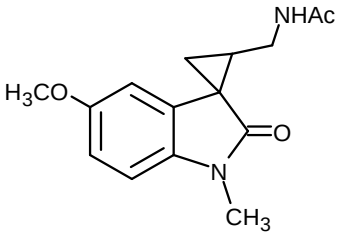

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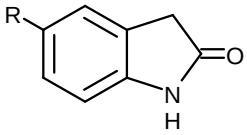
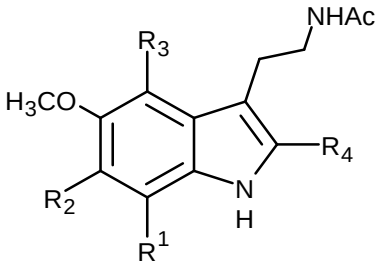


21: R = AcNH
22: R = NH_2

Figure 14: 2-Oxindole derivatives structures (**15–22**).

In 2018, Reina et al. [66] used a computer-assisted protocol to perform a systematic rational search for the design of newly MLT derivatives. Indeed, a total of 116 derivatives were originated through the addition of functional groups (i.e., -OH, -NH_2, -SH and -COOH) to the MLT structure. Among them, 20 MLT derivatives were expected to be the most promising, regarding drug-like behaviour. Furthermore, diverse reactivity indices were estimated, as well as their pK_a values. According to the gathered data, five MLT derivatives have been identified as the most likely candidates to act as chemical antioxidant (directly scavenging free radicals, by electron transfer and/or H transfer) (Figure 15).



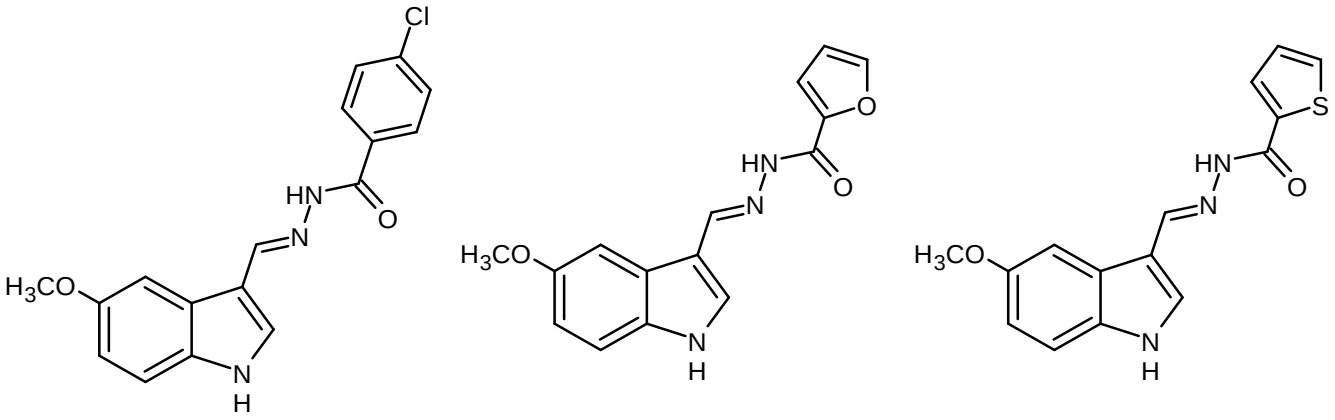
23: R_1 = SH, R_3 = OH, R_2 = R_4 = H
24: R_1 = SH, R_2 = OH, R_3 = R_4 = H,
25: R_1 = OH, R_2 = SH, R_3 = R_4 = H,
26: R_1 = R_3 = H, R_2 = SH, R_4 = COOH,
27: R_1 = SH, R_2 = R_3 = OH, R_4 = H

Figure 15: Designed MLT derivatives structures (**23–27**).

The antioxidant capacity of these five compounds was further evaluated by the research group in 2020 using the density functional theory [67]. Since acid–base equilibria regulate the antioxidant behaviour and are controlled by pH, they evaluated the molar fractions (Mf) of compounds at physiological pH. According to the Mf values, mono-anions are the most abundant species of the MLT derivatives, under such conditions. On the contrary, neutral MLT is the only species which occurs significantly at this pH. They assessed that these derivatives react with hydroperoxyl radicals at rates limited by aqueous solution, at physiological pH, while in lipid medium the reactions are slow. By contrast, in both media, these compounds exhibited a higher antioxidant effect than Trolox, ascorbic acid, resveratrol, and melatonin itself. The research group also evaluated the regeneration of MLT derivatives, after free radical attack, by reductants present in biological environments. The prediction indicated that only compound **26** (Figure 15) could be regenerated by $O_2 \cdot -$ to a significant extent. This aspect could improve the antioxidant capacity of this compound.

In 2020, Tchekalarova et al. [68] selected, among a series of previously synthesized indole derivatives, endowed with anticonvulsant activity and poor neurotoxicity and hepatotoxicity in rodents, the three most potent C3-modified derivatives with hydrazine structure (Figure 16) bearing 2-chlorophenyl (**28**), 2-furyl (**29**), and 2-thienyl (**30**) fragments, to evaluate their neurobiological activity in mice. The dose-dependent anxiolytic effect was studied in the open field test, while the

analgesic effect was tested in the hot plate test and formalin test. The antidepressant activity was evaluated by means the forced swimming test and tail suspension test-induced effect on markers of oxidative stress in the frontal cortex and the hippocampus. The three MLT analogues demonstrated improved antidepressant-like activity compared to the MLT, being devoid of anxiolytic effects. The antioxidant activity of the MLT analogues and analgesic potential was comparable to that of MLT. The research group corroborated that the 3C substitution with hydrazide/hydrazone moiety markedly provides the antidepressant and antioxidant activity of the MLT analogues. Furthermore, they hypnotized that the antidepressant and analgesic effects involved MT_1 receptors.



28 **29** **30**

Figure 16: Hydrazide indole derivatives structures (**28–30**).

In 2020, Panyatip et al. [69] reported five *N*-amide substituted MLT derivatives (Figure 17) whose scavenging potentials were screened by X-band electron spin resonance (ESR) and then comparatively evaluated by *in vitro* antioxidant assays such as hydroxyl radicals scavenging test, oxygen radical absorbance capacity (ORAC), 2,20 -azinobis(3-ethylbenzothiazoline-6-sulfonic acid) disodium salt (ABTS) and ferric reducing antioxidant power (FRAP) assays. Compounds **32–35** showed higher ESR response than MLT. The authors observed that addition of lipophilic groups probably hindered the electron transfer ability and reducing power of the compounds since the N1-substituted derivatives (**31–34**) presented lower activity than MLT in both the ABTS and FRAP assays. However, 4-bromobenzoyl and naphthoyl derivatives (**33** and **34**) showed a significant

hydroxyl radical inhibitory effect. All aryl derivatives (**32–35**) demonstrated an improved capability to quench peroxyl radicals than MLT about three times. The most powerful ability, assessed via oxygen radical absorbance capacity (ORAC) assay, was detected for benzoylated derivatives (**32** and **33**).

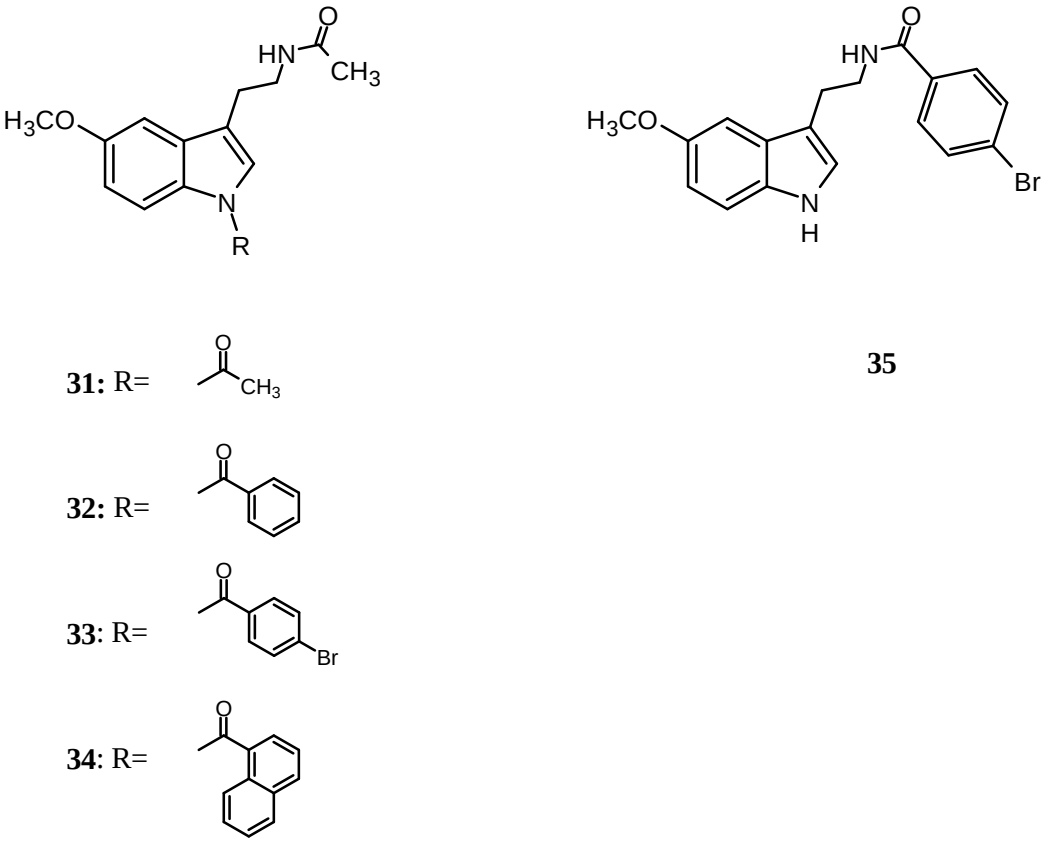
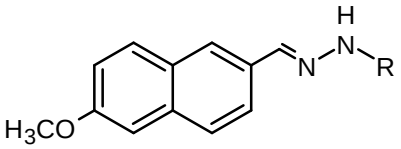


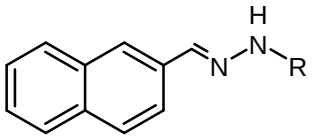
Figure 17: *N*-amide substituted MLT derivatives structures (**31–35**).

Recently, Shirinzadeh et al. [70] continued their studies on MLT analogues widening the structure-activity relationship of their compounds by replacing the indole nucleus of MLT with the bioisosteric naphthalene ring (Figure 18). The radical scavenging activities of the novel compounds were investigated by the DPPH assay. The authors found that the 1-(halogenated phenyl)-2-((6-methoxynaphthalen-2-yl)methylene)hydrazine derivatives (**36a–j**) exhibited a higher antioxidant effect than the 1-(halogenated phenyl)-2-naphthalen-2-yl)methylene)hydrazine (**36k–u**) derivatives, probably due to the 6-methoxy substitution process in these halogen bearing derivatives. In the halogen-free derivatives, methoxy substitution (**36i**, **36j**) was not found to cause an increase in

activity in comparison to the non-methoxy-substituted derivatives (**36t**, **36u**). The presence of halogen in the o-position reduced the radical scavenging activity (**36a**, **36d**, **36l** and **36o**), but *m*- and *p*-halogen substitution increased the activity of the corresponding compounds. The *in vitro* cytotoxic effects of the synthesized compounds were also investigated in CHO-K1 cells using the MTT assay, since some substances that are found to have radical scavenging effects appear to have cytotoxic potency. Results indicated that there was no significant structure-activity relationship in the potentially cytotoxic effects of the substances, but the 6-methoxy naphthalene compounds seemed to be more cytotoxic than the non-methoxy naphthalene compounds. Moreover, the ortho halogenation on the phenyl ring was found to decrease the cytotoxic potential of the compounds in general.



36a–j



36k–u

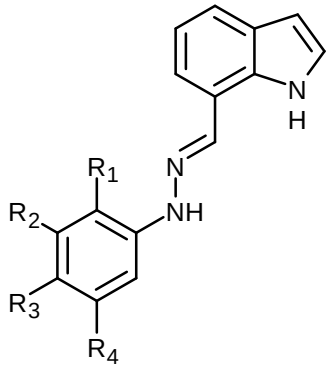
36a: R = 2-BrPh
36b: R = 3-BrPh
36c: R = 4-BrPh
36d: R = 2-ClPh
36e: R = 3-ClPh
36f: R = 4-ClPh
36g: R = 4-FPh
36h: R = 2,4-diMePh
36i: R = 3,4-diMePh
36j: R = Ph

36k: R = 2-BrPh
36l: R = 3-BrPh
36m: R = 4-BrPh
36n: R = 2-ClPh
36o: R = 3-ClPh
36p: R = 4-ClPh
36q: R = 3-FPh
36r: R = 4-FPh
36s: R = 2,4-diMePh
36t: R = 3,4-diMePh
36u: R = Ph

Figure 18: Bioisosteric naphthalene MLT analogues structures (**36a**–**u**).

Subsequently, in 2020, the same research group [71] prepared a series of new indole-7-aldehyde hydrazide/hydrazone derivatives (Figure 19) in which chemically significant modifications of the lead structure were made at two main positions of MLT molecule. Firstly, the methoxy group at the 5-position of the indole ring was eliminated and secondly side chain including formation of imine was made on the 7-position of the indole ring. They analysed the anticancer potential of the

compounds both by their antioxidant and CYP1 inhibitory activities. *In vitro* antioxidant capacity of the compounds was investigated both in a cell-based (DCFH assay) and a cell-free (DPPH assay) assay. Data indicated that *p*-halogenated derivatives (**37d**, **37n**) possessed the most powerful reducing and scavenging activity against reactive species among all tested mono-halogenated derivatives. On the contrary, bromo-substituted compound (**37i**) displayed prooxidative effect in DCFH assay as well as having the lowest radical scavenging activity in DPPH assay. Since human CYP1 enzymes is responsible for the catalysis of the metabolic activation of procarcinogens to ultimate carcinogens, the research group investigated the newly synthesized indole derivatives are their possible CYP1 inhibitory activity. All the tested compound showed a significant inhibition toward CYP1 enzymes. All newly synthesized indole based MLT analogues were found to have no cytotoxicity at their tested concentration.

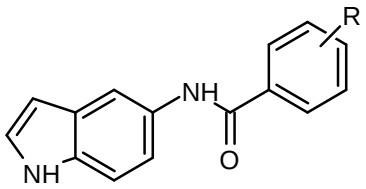


37a–u

37a: $R_1 = R_2 = R_3 = R_4 = H$
37b: $R_1 = Cl, R_2 = R_3 = R_4 = H$
37c: $R_1 = H, R_2 = Cl, R_3 = R_4 = H$
37d: $R_1 = R_2 = H, R_3 = Cl, R_4 = H$
37e: $R_1 = Cl, R_2 = H, R_3 = Cl, R_4 = H$
37f: $R_1 = Cl, R_2 = R_3 = H, R_4 = Cl$
37g: $R_1 = H, R_2 = R_3 = Cl, R_4 = H$
37g: $R_1 = H, R_2 = R_3 = Cl, R_4 = H$
37i: $R_1 = Br, R_2 = R_3 = R_4 = H$
37j: $R_1 = H, R_2 = Br, R_3 = R_4 = H$
37k: $R_1 = R_2 = H, R_3 = Cl, R_4 = H$
37l: $R_1 = F, R_2 = R_3 = R_4 = H$
37m: $R_1 = H, R_2 = F, R_3 = R_4 = H$
37n: $R_1 = R_2 = H, R_3 = F, R_4 = H$
37o: $R_1 = F, R_2 = H, R_3 = F, R_4 = H$
37p: $R_1 = F, R_2 = R_3 = H, R_4 = F$
37q: $R_1 = H, R_2 = F, R_3 = H, R_4 = F$
37r: $R_1 = CH_3, R_2 = H, R_3 = CH_3, R_4 = H$
37s: $R_1 = H, R_2 = CH_3, R_3 = CH_3, R_4 = H$
37t: $R_1 = R_2 = H, R_3 = OCH_3, R_4 = H$
37u: $R_1 = R_2 = H, R_3 = CN, R_4 = H$

Figure 19: Indole-7-aldehyde hydrazide derivatives structures (**37a–u**).

In 2021 Elkamhawy et al. [72] synthesized a series of MLT analogues as novel monoamine oxidase B (MAO-B) inhibitors with the potential to counteract oxidative stress in neuronal PC12 cells. Indeed MAO-B metabolizes dopamine and plays an important role in oxidative stress by altering the redox state of neuronal and glial cells. Among them, **38n**, **38r** and **38u–w** (Figure 20) demonstrated >70% inhibition of MAO-B at 10 μM with IC_{50} values of 1.41, 0.91, 1.20, 0.66 and 2.41 μM, respectively. Moreover, these compounds displayed a better selectivity index versus MAO-A, when compared with rasagiline (a well-known MAO-B inhibitor). Additionally, compounds **38n** and **38r** demonstrated a safe toxicity profile and effectively reduced 6-OHDA- and rotenone-induced oxidative toxicity in PC12 cells through the up-regulation of the Nrf2/HO-1 signalling pathway. Therefore, the authors proposed compounds **38n** and **38r** as new multi-targeted candidates worthy of further development for oxidative stress-related PD therapy.



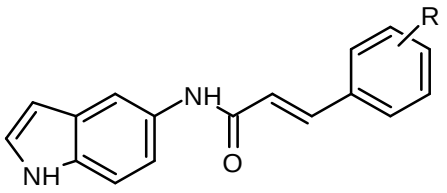
38a–x

38a: R = H
38b: R = 2-Br
38c: R = 4-Br
38d: R = 2,4-diCl
38e: R = 3,5-diNO_2
38f: R = 3-NO_2
38g: R = 4-NO_2
38h: R = 2,6-diF
38i: R = 3-F
38j: R = 4-F
38k: R = 2-F
38l: R = 2-CF_3, 4-F
38m: R = 3-CN
38n: R = 3-Cl
38o: R = 2-Cl
38p: R = 4-Cl
38q: R = 4-*tert*-butyl
38r: R = 3-Br
38s: R = 3-OCF_3
38t: R = 4-OCF_3
38u: R = 3-CF_3
38v: R = 4-CF_3
38w: R = 3,5-diCl
38x: R = 2,6-diCl

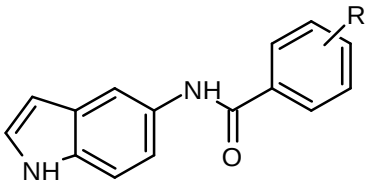
Figure 20: Indole-based MLT analogues structures (**38a–x**).

More recently a structure-based hybridization of two natural antioxidants (MLT and caffeic acid) afforded a novel hybrid series of indole-based amide analogues (**39a–d**) which performed as potent

free radical scavenging agents more active than their benzamide analogues (**39e–m**) (Figure 21) [73]. Compared to Trolox, a water-soluble analogue of vitamin E, compounds **39b–e** and **39j** were found to have excellent DPPH radical scavenging activities. Three compounds out of five **39b–d** showed a higher capacity to neutralize the radical cation $ABTS^{\bullet+}$ more than Trolox. Using FRAP and ORAC assays, compound **39c** was the most active antioxidant agent that has been reported by the authors as a new antioxidant candidate for the treatment of oxidative stress-related diseases.



39a–d



39e–m

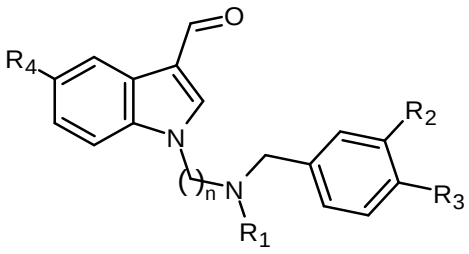
39a: R = 4-OH
39b: R = 3-OMe, 4-OH
39c: R = 3,4-diOH
39d: R = 3,5-diOMe, 4OH

39e: R = 3-OH,4-NH_2
39f: R = 2-Br, 4,5-diOMe
39g: R = 4-Br, 3,5-diOH
39h: R = 3-NH_2, 4-OH
39i: R = 3,5-diOMe, 4-OH
39j: R = 3,4-diOH
39k: R = 2-Me, 4-OH
39l: R = 4-OH
39m: R = 3-OMe, 4-OH

Figure 21: Indole-based caffeic acid amides (**39a–d**) and their benzamide analogues (**39e–m**).

Also, in 2023 a series of MLT-alkylbenzylamine hybrids as multitarget agents for the treatment of Alzheimer's disease has been reported (Figure 22) [74]. Compounds by Liu et al. exhibited a potent multifunctional profile involving cholinesterase inhibition and antioxidant effects. Almost all the compounds displayed moderate butyrylcholinesterase (BuChE) inhibitory activities with IC_{50} values in the submicromolar to micromolar level. In addition, most compounds showed more effective inhibition for BuChE than for acetylcholinesterase (AChE). Among them, compound **40m** (n: 6, R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe) showed the most potent inhibitory activity for AChE (IC_{50} = 0.54 ± 0.09 μM). Compound **40v** n: 12, R_1 = Et, R_2 = OH, R_3 = H, R_4 = OMe) exhibited the most potent

inhibitory activity for BuChE (IC_{50} = 0.19 ± 0.01 μM) and compound **40i** (n: 12, R_1 = Me, R_2 = OH, R_3 = H, R_4 = OMe) had the highest selectivity towards BuChE versus AChE (IC_{50} (AChE)/IC_{50} (BuChE) = 87.38). Among them, compounds **40j** (n: 3, R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe), **40n** (n: 7, R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe) and **42** (n: 12, R_1 = Me, R_2 = OH, R_3 = H, R_4 = OH) exhibited potent peroxyl radical absorbance capacities (ORAC > 1). The authors underlined that the presence of phenolic groups greatly increased the free radical scavenging ability of the compounds. Indeed, compounds **40s–u** without phenolic group did not exhibit potent peroxyl radical absorbance capacities. Among these analogues, compound **42** exerted an excellent selective for BuChE, the most potent free radicals scavenging capacity as well as good ability to penetrate the BBB. Furthermore, compound **42** promote the translocation of Nrf2 to the nucleus and increase expression level of Nrf2 and its target. Therefore, the authors proposed compound **42** as a promising candidate to be further developed for the therapy of AD treatment.



40a–v,41,42

40a–i: n: 3–10,12, R_1 = Me, R_2 = OH, R_3 = H, R_4 = OMe
40j–r: n: 3–10,12, R_1 = Me, R_2 = H, R_3 = OH, R_4 = OMe
40s: n: 12, R_1 = Me, R_2 = OMe, R_3 = H, R_4 = OMe
40t: n: 12, R_1 = Me, R_2 = H, R_3 = OMe, R_4 = OMe
40u: n: 12, R_1 = Me, R_2 = OMe, R_3 = OMe, R_4 = OMe
40v: n: 12, R_1 = Et, R_2 = OH, R_3 = H, R_4 = OMe
41: n: 14, R_1 = Me, R_2 = OH, R_3 = H, R_4 = OMe
42: n: 12, R_1 = Me, R_2 = OH, R_3 = H, R_4 = OH

Figure 22: MLT-alkylbenzylamine hybrids (**40a–v,41,42**).

Table 2 summarized the antioxidant activities of MLT synthetic analogues and related biological assays to assess these activities.

Table 2. Antioxidant activity of MLT synthetic analogues.

Compounds	Activity	Assay	Reference
1b, **1c**, **1m**, **1k** and **1l**	Antioxidant activity, protective effect on H_2O_2-induced malondialdehyde formation in erythrocyte membranes, protective effect against AAPH-induced oxidative hemolysis	*In vitro: e*stimation of Reactive Oxygen Species (ROS) by 2',7'-dichlorodihydrofluorescein diacetate assay (DCFH-DA), measurement of H_2O_2-induced lipid peroxidation levels, determination of Erythrocyte Hemolysis	Shirinzadeh et al. [55]
2	Scavenging activity against the DPPH radical, inhibitory effect on the superoxide radical scavenging assay	*In vitro:* DPPH free radical scavenging activity, Superoxide radical scavenging activity	Yılmaz et al. [56]
3	Antioxidant activity, inhibitory effects on lipid peroxidation	*In vitro:* DPPH free radical scavenging activity, lipid peroxidation assay	Suzen et al. [57]
4g, **4l**, and **4n**	Protective effect of DCFH-DA in human Erythrocytes, decrease of LDH leakage	*In vitro: e*stimation of ROS by DCFH-DA assay, lactate dehydrogenase leakage assay, electrochemical measurements	Suzen et al. [58]
5b, **5h**, and **5i**	Antioxidant capacity in the DPPH and superoxide radical scavenging assays	*In vitro:* DPPH free radical scavenging activity, Superoxide radical scavenging activity	Karaaslan et al. [59]
6b, **6c**	Antioxidant activity, Ca^{2+}/calmodulin-dependent kinase II inhibitor	*In vitro: e*stimation of ROS by DCFH-DA assay, Ca^{2+}/calmodulin-dependent kinase II inhibition assay	Carocci et al. [60]
10a	Antioxidant activity	*In vitro: e*stimation of ROS by DCFH-DA assay	Puskullu et al. [62]
11b and **12e**	Antioxidant activity	*In vitro:* DPPH free radical scavenging activity, *e*stimation of ROS by DCFH-DA assay	Puskullu et al. [63]
14a, **14h**, **14j** and **14m**	Antioxidant activity, cell damages protection	*In vitro: e*stimation of ROS by DCFH-DA assay on human erythrocytes, lactate dehydrogenase leakage assay on CHO-K1 cells, MTT assay on neuronal PC12 cells	Gurer-Orhan et al. [64]

15, 21, 22	Compound **15**: hypotensive effect by reducing IOP *in vivo*; Compounds **21**,**22**: high antioxidant activity	*In vivo*: evaluation of the ability to reduce the intraocular pressure (IOP) on normotensive male Chinchilla rabbit; *in vitro*: evaluation of the antioxidant activity by changes in parameters of chemiluminescence kinetics in hemoglobin−H_2O_2−luminol model system	Chesnokova et al. [65]
23–27	Identified as the most likely candidates to act as chemical antioxidant (directly scavenging free radicals, by electron transfer and/or H transfer)	*In silico*: estimation of physicochemical parameters such as absorption, distribution, metabolism and excretion (ADME) properties using Molinspiration Property Calculation Service and DruLiTo software	Reina et al. [66]
26	Antioxidant activity	*In silico:* evaluation of the molar fractions (Mf) of compounds at physiological pH	Castañeda-Arriaga et al. [67]
28–30	Antidepressant-like activity, antioxidant activity, analgesic effect	*In vivo: t*est for anxiety in open field test, evaluation of the antidepressant activity by means the forced swimming test and tail suspension test-induced effect on markers of oxidative stress in the frontal cortex and the hippocampus, detection of oxidative stress in mouse homogenates	Tchekalarova et al. [68]
31-35	Antioxidant activity	*In vitro:* hydroxyl radicals scavenging test, oxygen radical absorbance capacity (ORAC), 2,20 -azinobis(3-ethylbenzothiazoline-6-sulfonic acid) disodium salt (ABTS) and ferric reducing antioxidant power (FRAP) assays, Electron Spin Resonance (ESR) Study	Panyatip et al. [69]
36a–j	Antioxidant activity	*In vitro:* DPPH free radical scavenging activity	Shirinzadeh et al. [70]

37d, 37n	Antioxidant activity, inhibition toward CYP1 enzymes	*In vitro*: DPPH free radical scavenging activity, *e*stimation of ROS by DCFH-DA assay, evaluation of CYP1 inhibitory activity	Shirinzadeh et al. [71]
38n, 38r and **38u–w**	Monoamine oxidase B (MAO-B) inhibitors, reduced 6-OHDA- and rotenone-induced oxidative toxicity in PC12 cells, up-regulation of the Nrf2/HO-1 signalling pathway, ability to prevent LPO induced by Fe^{2+} and L-ascorbic acid in rat brain homogenates	*In vitro:* cell viability measurements by MTT assay, western blotting analysis, monoamine oxidase (MAO) enzyme assay; *In vivo*: assessment of lipid peroxidation (LPO) in rat brain homogenates	Elkamhawy et al. [72]
39b–e and **39j**	Antioxidant activity	*In vitro:* DPPH free radical scavenging activity, hydroxyl radicals scavenging test, oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP) assays	Elkamhawy et al. [73]
40m, 40v, 40i, 40j, 40n and **42**	**40m**: inhibitory activity on AChE; **40v, 40i, 42**: inhibitory activity on BuChE; **40j**, **40n** and **42**: peroxyl radical absorbance capacities. **42**: ability to penetrate the BBB, promote the translocation of Nrf2 to the nucleus and increase expression level of Nrf2 and its target	*In vitro*: inhibitory activities on butylcholinesterase (BuChE), acetylcholinesterase (AChE), S-butylthiocholine iodide (BTCI), acetylthiocholine iodide (ATCI) and 5,5-dithiobis-(2-nitrobenzoicacid) (DTNB), oxygen radical absorbance capacity (ORAC), PAMPA-BBB penetration assay, Western blot analysis	Liu et al. [74]

3. Conclusions

MLT, originally discovered as a hormone of the pineal gland and responsible for the regulation of the sleep/wake cycle, has been extensively studied for its pleiotropic effects. Indeed, MLT possesses a plethora of activities that make it remarkably efficacious in decreasing the subcellular turmoil

promoted by oxidative destruction of key cellular elements which, when damaged, compromise the optimal function of cells often resulting in their disintegration via apoptosis or necrosis. MLT has redox properties because of the presence of an electron-rich aromatic ring system, which allows the indoleamine to easily function as an electron donor. Conceivably, making the indole ring more stable electronically could help to act as a better electro donor. Due to MLT unique antioxidant potential, research efforts have been focused on the design, synthesis, and study of MLT derivatives that could improve the antioxidant profile and the already promising activities of the parent molecule. The common strategy consists of modifying the groups in the different sites of the indole ring or replacing it with a bioisosteric one. Several studies have shown that the lack of the methoxy group does not significantly affect the antioxidant capacity of the MLT analogues, indeed several compounds with modifications in the methoxy and acylamino ethyl chain were identified as better antioxidants than MLT itself. It was thought that the introduction of an imine group into the side chain increased the stability of the indole molecule by helping the delocalization of the electrons and this might help to have high free radical scavenging activity in the synthesized compounds. Indeed, the combination of indole nucleus with hydrazone/hydrazide-type compounds containing aromatic halogenated rings increased the antioxidant activity of indoles compared to MLT, thus providing new effective drugs against free radicals. In this review, we examined the current state of the art in the development of new MLT analogues as antioxidant, also dwelling on the *in vivo* studies. Expectations about the new MLT-related compounds under investigation are high, therefore, their continuous study and optimization should be envisaged, that could lead in the future to the realization of valid therapeutic alternatives in treating oxidative stress-related disorders.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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