Short notes

3-(3,4-Dichlorophenyl)-5-(1*H*-indol-5-yl)-1,2,4-oxadiazole

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Abstract: 3-(3,4-Dichlorophenyl)-5-(1*H*-indol-5-yl)-1,2,4-oxadiazole was synthesized *via* the condensation of 3,4-dichlorobenzamidoxime and methyl 1*H*-indole-5-carboxylate using superbasic medium (NaOH/DMSO). The compound was tested as a potential inhibitor of human monoamine oxidase (MAO) A and B. It demonstrated notable inhibition with an IC_{50} value of 0.036 μM for MAO-B and isoform specificity. The product was characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. In conclusion, the new active MAO-B inhibitor may serve as a candidate for the future discovery of therapeutic agents for neurodegenerative disorders such as Parkinson's disease.

Keywords: monoamine oxidase; inhibitors; indole; 1,2,4-oxadiazole; drug research; neuroprotective drugs; Parkinson's disease; MAO

1. Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disorder of the central nervous system (CNS), currently affecting over 8% of individuals aged 65 years and older worldwide. PD results in the chronic, irreversible, and progressive neuronal degradation of specific areas in the human brain and is caused by complex pathophysiological processes, including oxidative stress, neuro-inflammation, excitotoxicity, mitochondrial dysfunction, and proteolytic stress [1–3].

Monoamine oxidases (MAOs) are mitochondrial flavoenzymes that play a key role in the metabolism of monoaminergic neurotransmitters. The selective inhibition of MAO-B is a well-established approach in the treatment of PD [4-6]. For example, the irreversible MAO-B inhibitor, selegiline, and the reversible inhibitor, safinamide, have been approved for the treatment of PD [4-6] (Figure 1a).

Despite the diversity of MAO-B inhibitors that has been described in literature, the discovery of novel inhibitors with good potencies and isoform specificities is still of interest. Recently, indole and indazole derivatives have been reported to be highly potent and specific MAO-B inhibitors (Figure 1b) [7]. However, the amide bond in the central fragment of these compounds may be metabolically labile and could lead to rapid inactivation of future drugs in this class.

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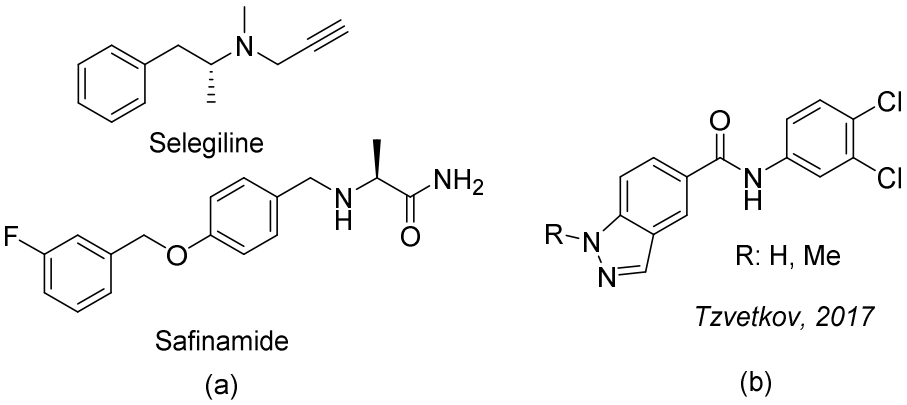


Figure 1. (**a**) Structures of MAO-B specific inhibitors; (**b**) The series of indole and indazole derived MAO inhibitors.

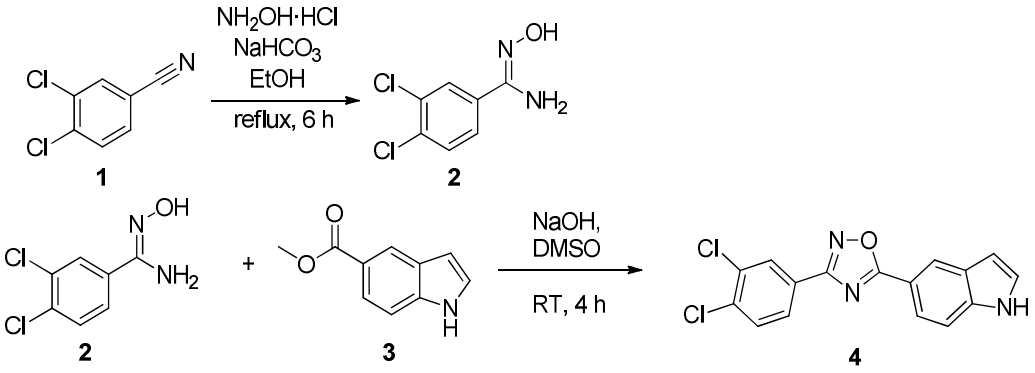
Our research group has reported a variety of new lead compounds for the discovery of isoform specific MAO-B inhibitors such as imidazolines, benzenesulfonamides and pyrazolo[1,5-*a*]quinoxalin-4-ones [8-10]. Based on our interest in the discovery of novel MAO-B inhibitors, in this work we used our previously developed synthetic approach to synthesize new a indole-derivative where the amide functionality has been replaced by a bioisostere.

According to data of the Swiss Institute of Bioinformatics, the central amide group of the molecule can be replaced without loss of biological activity by the 1,2,4-oxadiazole heterocyclic fragment. This substitution should have a favorable effect on the *in vivo* metabolic stability of drug leads due to the greater stability of fragments of five-membered heterocycles compared to the amide bond.

2. Results

2.1. Chemistry

3-(3,4-Dichlorophenyl)-5-(1*H*-indol-5-yl)-1,2,4-oxadiazole (**4**) was synthesized by the condensation of amidoxime **2** with carboxylic acid ester **3** in the superbasic medium (NaOH/DMSO) as described in our previous work [11]. The amidoxime **2** was synthesized from the corresponding nitrile **1** as described in literature [12].



Scheme 1. Synthesis of 3,4-dichlorobenzamidoxime **2** and 3-(3,4-dichlorophenyl)-5-(*1*H-indol-5-yl)-1,2,4-oxadiazole **4**.

2.2. MAO inhibition

The MAO inhibition potency of 3-(3,4-dichlorophenyl)-5-(1*H*-indol-5-yl)-1,2,4-oxadiazole was investigated using recombinant human MAO-A and MAO-B, following the same protocol as described for the previous investigation of 1,3,4-oxadiazol-2-ylbenzene-

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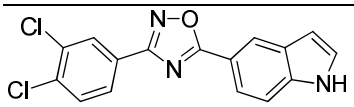
sulfonamides [8]. The results of the MAO inhibition studies are presented in table 1. Compound **4** inhibited MAO-B with an IC_{50} value of 0.036 μM, whereas weak inhibition was recorded for MAO-A.

Table 1. The inhibition of human MAO-A and MAO-B by 3-(3,4-dichlorophenyl)-5-(1*H*-indol-5-yl)-1,2,4-oxadiazole **4**.

Structure	IC_{50} (μM ± SD)[1]	
	MAO-A	MAO-B
	150 ± 7.88	0.036 ± 0.012
Safinamide[2]	-	0.048
Toloxatone[2]	3.92	-

[1] The IC_{50} values are presented as the means ± standard deviation (SD) of triplicate measurements
[2] Reference inhibitors [13]

3. Discussion

This study reports the MAO inhibition potency of 3-(3,4-dichlorophenyl)-5-(1*H*-indol-5-yl)-1,2,4-oxadiazole (**4**). This compound inhibited MAO-B with an IC_{50} value of 0.036 μM while the MAO-A isoform was inhibited with an IC_{50} value of 150 μM. The discovery of this active MAO-B inhibitor paves the way for the future discovery of potent MAO-B inhibitors among indole derivatives, which may find application in the treatment of neurodegenerative disorders such as PD.

4. Materials and Methods

4.1. General

All and reagents and solvents were obtained from commercial sources and were used without purification. Reactions were monitored by analytical thin layer chromatography (TLC) using Silufol-254 plates. Visualization of the developed plates was performed by fluorescence quenching at 254 nm. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian 400 Unity Plus instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively). Chemical shifts (δ) are given in parts per million (ppm) and were referenced to the solvent signal for DMSO-*d*6 (2.50 for proton and 39.52 for carbon), while the coupling constants (J) are reported in hertz (Hz). Multiplicities are abbreviated as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. Melting points were determined on an Electrothermal IA 9300 series digital melting point apparatus. Mass spectra were recorded on microTOF spectrometers (ESI ionization).

4.2. Synthesis and characterization of 3-(3,4-dichlorophenyl)-5-(1H-indol-5-yl)-1,2,4-oxadiazole 4

To a solution of 3,4-dichlorobenzamidoxime **2** (0.0015 mol, 1 equiv.) and methyl 1*H*-indole-5-carboxylate **3** (0.0015 mol, 1 equiv.) in DMSO (1 mL), powdered NaOH (0.002 mol, 1.3 equiv.) was rapidly added. The reaction mixture was stirred at room temperature for the required time (TLC). The reaction mixture was diluted with cold water (30-50 mL). The resulting precipitate was collected by filtration, washed with water (30 mL) and air-dried at 50 °C. Yield 0.391g, 79%, beige solid, mp 193-195 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.64 (s, 1H), 8.45 (s, 1H), 8.21 (d, J = 2.1 Hz, 1H), 8.03 (dd, J = 8.4, 2.0 Hz, 1H), 7.91 – 7.82 (m, 2H), 7.62 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 3.2 Hz, 1H), 6.66 (d, J = 3.2 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 178.05, 167.05, 139.17, 134.86, 132.79, 132.32, 129.32, 128.49, 127.74, 122.09, 121.25, 114.51, 113.17, 103.41; MS (ESI^{+}): *m/z* $[M+H]^{+}$. Anal. Calcd for $C_{16}H_{10}Cl_2N_3O$: 330.0196. Found: 330.0182.

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4.3. Synthesis and characterization of 3,4-dichlorobenzamidoxime 2 [14]

To a suspension of 3,4-dichlorobenzonitrile (0.012 mol, 1 equiv.) and $NH_2OH{\cdot}HCl$ (0.018 mol, 1.5 equiv.) in ethanol (15 ml), $NaHCO_3$ (0.018 mol, 1.5 equiv.) was added. The reaction mixture was heated under reflux for 2 h (TLC). The solvent was subsequently evaporated under reduced pressure, and the reaction mixture was diluted with cold water. The resulting precipitate was collected by filtration, washed with water (30 mL) and air-dried at 50 °C. Yield 86%, white solid, mp 143-145 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.87 (s, 1H), 7.89 (d, J = 1.9 Hz, 1H), 7.71 – 7.60 (m, 2H), 5.95 (s, 2H).

4.4. MAO inhibition studies

The measurement of IC_{50} values for the inhibition of human MAO-A and MAO-B was carried out according to previously reported protocols [8, 15]. Recombinant human MAO-A and MAO-B were obtained from Sigma-Aldrich and fluorescence measurements were rescored with a SpectraMax iD3 Multi-Mode microplate reader (Molecular Devices). The measurement of MAO activity was based on the fluorescence signal generated when the substrate, kynuramine, is oxidized by the MAOs to yield 4-hydroxyquinoline.

Supplementary Materials: Copies of 1H and 13C NMR spectra.

Author Contributions: The following statements should be used "Conceptualization Conceptualization of the study was done by Anton Shetnev and Sergey Baykov; formal analysis was done by Sergey Baykov.; formal analysis and investigation - Julia Efimova and Anél Petzer; writing—original draft preparation was done by Julia Efimova; writing—review and editing was done by Anton Shetnev and Jacobus P.Petzer. All authors have read and agreed to the published version of the manuscript."

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