

Communication

Synthesis and Monoamine Oxidase Inhibition Properties of 4-(2-Methyloxazol-4-yl)benzenesulfonamide

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Abstract: 4-(2-Methyloxazol-4-yl)benzenesulfonamide was synthesized by the reaction of 4-(2-bromoacetyl)benzenesulfonamide with an excess of acetamide. The compound was evaluated as a potential inhibitor of human monoamine oxidase (MAO) A and B and was found to inhibit these enzymes with IC₅₀ values of 43.3 and 3.47 μM, respectively. The potential binding orientation and interactions of the inhibitor with MAO-B were examined by molecular docking, and it was found that the sulfonamide group binds and interacts with residues of the substrate cavity. 4-(2-Methyloxazol-4-yl)benzenesulfonamide showed no cytotoxic effect against human stromal bone cell line (HS-5) in the concentration range of 1–100 μmol. Thus, the new selective MAO-B inhibitor was identified, which may be used as the lead compound for the development of antiparkinsonian agents.

Keywords: monoamine oxidase; MAO; inhibitor; 1,3-oxazole; benzenesulfonamide; drug research; Parkinson's disease; molecular docking



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1. Introduction

Neurodegenerative disorders significantly affect the health of the human population and place a burden on economies of countries around the world. Among these disorders, Parkinson's disease (PD) is the second most prevalent and is associated with the death of dopaminergic motor neurons in the brain. At present, PD is not curable; however, the motor symptoms associated with this disorder are effectively treated with levodopa, the metabolic precursor of dopamine. To enhance the therapeutic action of levodopa, this drug is frequently combined with monoamine oxidase (MAO) B inhibitors, compounds that reduce the MAO-mediated central metabolism of dopamine [1]. While MAO-B inhibitors may allow for a reduction in the effective levodopa dosage, these compounds have also been studied as neuroprotective agents [2], candidates for reducing neuroinflammation [3–6], as well as compounds with potential value in the therapy of oncological diseases [7]. Possible mechanisms by which MAO-B inhibitors may exert neuroprotective effects include the enhancement of the levels of brain-derived neurotrophic factors (BDNFs) [8] and the molecular adhesion of L1CAM (L1) nerve cells, which can also promote axonal regrowth and increase neuronal survival, synaptic plasticity, and remyelination [9]. Thus, the development of a new generation of neuroprotective agents that act by inhibiting MAO might have relevance in the future treatment of neurodegenerative disorders.

Benzenesulfonamide compounds have been identified as potent and isoform-specific inhibitors of MAO-B, with some compounds exhibiting potencies in the nanomolar range. Such compounds might represent promising candidates for the future treatment of PD [10,11]. Also, it has been established that 1,3-oxazole derivatives exhibit the potent and specific

inhibition of the MAO-B isoform [12]. This work reports a successful attempt to combine both these structural features into a single molecule (Figure 1).

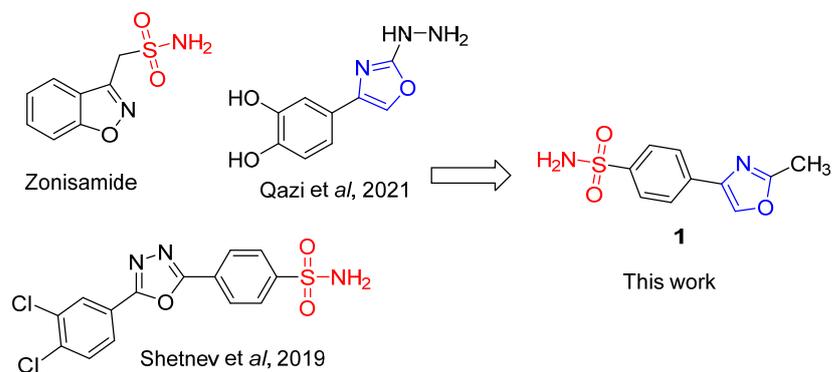


Figure 1. Structures of MAO inhibitors containing the sulfonamide or 1,3-oxazole moieties [11,12].

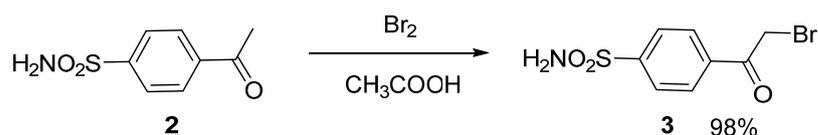
Recently, our research group, as well as others, reported a variety of new lead compounds for the development of isoform-specific MAO-B inhibitors, such as 2,1-benzisoxazoles [13,14], indazoles [15,16], and quinoxalines [17,18]. Continuing the search for novel MAO inhibitors, in this work, we used a simple convergent approach to synthesize a new 1,3-oxazole compound substituted with a primary benzenesulfonamide functionality.

2. Results

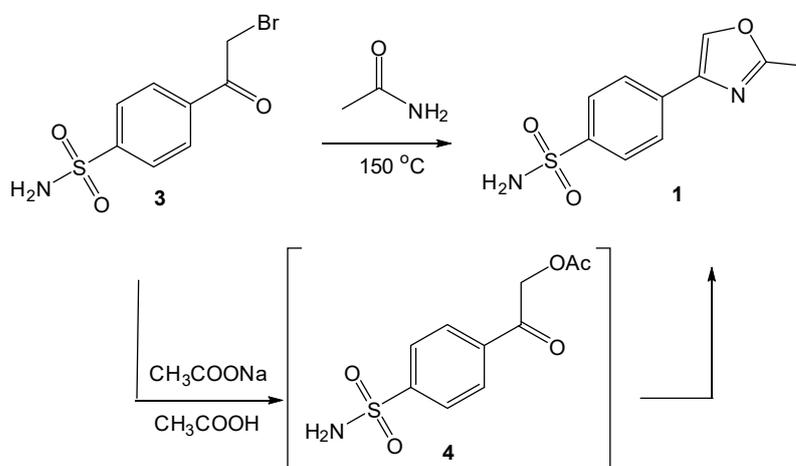
2.1. Chemistry

We investigated the possibility of synthesizing the target oxazole **1** by the reaction of sulfonamide-containing phenacyl bromide **2** with acetamide or ammonium acetate.

The starting material, phenacyl bromide **3**, was obtained according to a well-known three-step procedure using 4-acetylbenzenesulfonamide (**2**) as a key reagent (Scheme 1) [19,20]. The best yield (63%) of the target oxazole **1** was obtained by fusing phenacyl bromide **3** with an excess of acetamide at 150 °C (Scheme 2). It was shown that the reaction ends in 15–20 min, and longer heating or increasing the reaction temperature to 190 °C leads to a sharp decrease in the yield of **1** and the appearance of degradation by-products.



Scheme 1. Synthesis of 4-(2-bromoacetyl)benzenesulfonamide (**3**).



Scheme 2. Synthesis of 4-(2-methyloxazol-4-yl)benzenesulfonamide (**1**).

Additionally, when we used the method in [21] based on reaction compound 5 with ammonium acetate in acetic acid, the target oxazole 1 was obtained at a much lower yield (24%).

2.2. MAO Inhibition

The MAO inhibition potency of 4-(2-methyloxazol-4-yl)benzenesulfonamide (1) was evaluated using recombinant human MAO-A and MAO-B following the protocol described in the literature [13]. The results of the MAO inhibition studies are presented in Table 1. Compound 1 inhibited MAO-B with an IC_{50} value of 3.47 μ M, whereas weak inhibition of the MAO-A isoform was recorded.

The potential orientations by which 1 binds to the active site of human MAO-B was investigated by molecular docking using the CDOCKER module of the Discovery Studio 3.1 modeling software. Docking predicts that the inhibitor binds with the sulfonamide group placed in the substrate cavity of the enzyme, while the oxazole moiety extends towards the entrance cavity of the MAO-B active site (Figure 2).

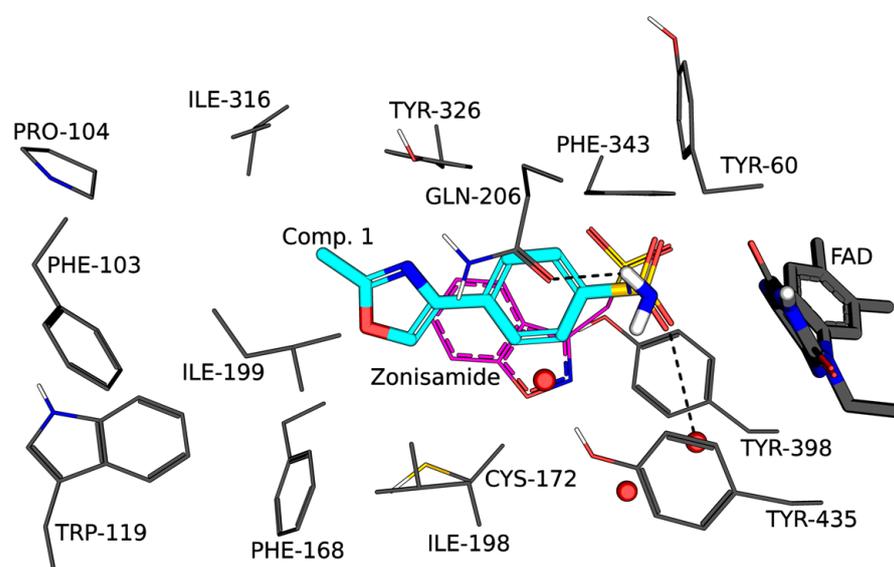
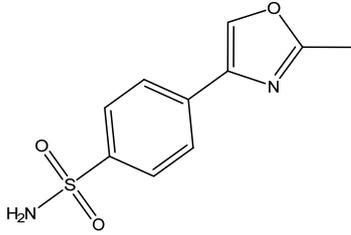


Figure 2. The predicted binding orientation of compound 1 to the active site of MAO-B. Zonisamide shown in pink, oxazole compound 1 shown in blue, red dots showing the binding regions to the target cavity.

The experimentally determined binding mode of zonisamide is also shown [22]. The sulfonamide group is placed in the same space as that of zonisamide, as determined by X-ray crystallography [22]. Hydrogen bonding occurs between the sulfonamide functional group and an active site water and Gln-206. Pi-sulfur interactions form between Tyr-60 and the sulfonamide functional group, as well between Cys-172 and the oxazole moiety. A pi-pi interaction also occurs between the oxazole moiety and Tyr-326. It is interesting to note that the oxazole moiety does not protrude deep into the entrance cavity and therefore cannot be viewed as a cavity-spanning MAO-B inhibitor. Compounds that bind to both the substrate and entrance cavities often exhibit submicromolar potencies due to the additional stabilization afforded by nonpolar interactions with the lipophilic environment of the entrance cavity [23]. This may explain the moderate MAO-B inhibition potency observed for compound 1.

Table 1. The inhibition of human MAO-A and MAO-B by 4-(2-methyloxazol-4-yl)benzenesulfonamide (1).

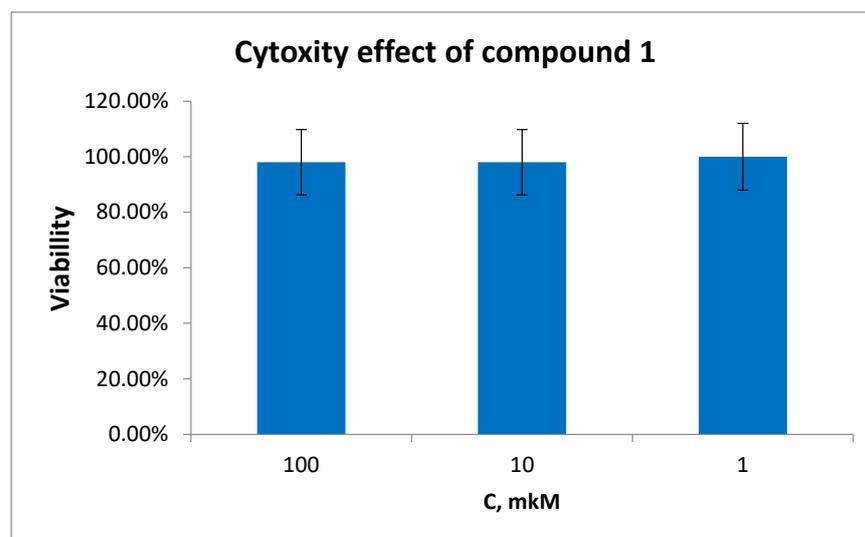
Structure	IC ₅₀ (μM ± SD) ¹	
	MAO-A	MAO-B
	43.3 ± 7.12	3.47 ± 0.31
Curcumin [24] ²	5.02 ± 0.45	2.56 ± 0.21
Toloxatone [25] ²	3.92	-

¹ The IC₅₀ values are presented as the means ± standard deviation (SD) of triplicate measurements. ² Reference inhibitors.

2.3. Cytotoxicity In Vitro

The cytotoxicity of compound **1** was investigated on human cell line HS-5 (human bone marrow stroma). In this respect, the cell viability after being incubated with 1–100 μM of the test compound was evaluated using the MTT assay [26,27].

The result of that experiment showed that compound **1** did not exhibit toxicity at the concentration range used for this study (Figure 3).

**Figure 3.** Cell viability after treatment of HS-5 stromal cell line with compound **1** (1–100 μM).

3. Discussion

This study reports the MAO inhibition potency of 4-(2-methyloxazol-4-yl)benzenesulfonamide (**1**). This compound inhibited MAO-B with an IC₅₀ value of 3.47 μM, while the MAO-A isoform was inhibited with an IC₅₀ value of 43.3 μM. Molecular docking experiments show that the inhibitor is restricted to the substrate cavity and does not extend deep into the entrance cavity, which may explain the moderate MAO-B inhibition potency observed for this compound. The discovery of this active MAO-B inhibitor paves the way for the future discovery of MAO-B inhibitors among 1,3-oxazole derivatives substituted with a primary benzenesulfonamide. Such compounds may find application in the treatment of neurodegenerative disorders such as PD.

4. Materials and Methods

4.1. General

All reagents and solvents were obtained from commercial sources (Aldrich, Merck, Aladdin, Gernsheim, Germany) and were used without purification. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck TLC sheets. Visualization of the developed sheets was performed by fluorescence quenching at 254 nm. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian 400 Unity Plus instrument (400 MHz for ^1H 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 ppm for proton and 39.52 ppm for carbon), while the coupling constants (J) were 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 using ESI ionization on a microTOF spectrometer (Bruker Daltonics Inc., Bremen, Germany).

4.2. Procedure for the Preparation of 4-(2-Bromoacetyl)benzenesulfonamide (3)

Compound **3** was prepared according to the method described in [20] using 0.90 g 4-acetylbenzenesulfonamide (**2**) as a starting material. (**3**) In total, 1.22 g (98%) of 4-(2-Bromoacetyl)benzenesulfonamide was isolated as the beige crystalline solid. mp 154–155 °C. ^1H NMR (400 MHz, DMSO-*d*₆) δ 8.17 (d, J = 8.2 Hz, 2H), 7.96 (d, J = 8.2 Hz, 2H), 7.57 (s, 2H), 4.98 (s, 2H).

4.3. Synthesis and Characterization of 4-(2-Methyloxazol-4-yl)benzenesulfonamide (1)

Acetamide (0.12 g, 2 mmol, 3 equiv.) and 4-(2-bromoacetyl)benzenesulfonamide (**3**; 0.19 g, 0.68 mmol, 1 equiv.) were mixed together. The reaction mixture was melted, stirred at 150 °C for 20 min, and subsequently diluted with cold water (30 mL). The resulting precipitate was collected by filtration, washed with water (10 mL), and air-dried at 50 °C. Yield 0.101 g, 63%, beige solid, mp 237–239 °C; ^1H NMR (400 MHz, DMSO-*d*₆) δ 8.61 (s, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.85 (d, J = 8.4 Hz, 2H), 7.38 (s, 2H), 2.48 (s, 3H); ^{13}C NMR (101 MHz, DMSO-*d*₆) δ 162.6, 143.7, 139.2, 137.0, 134.9, 126.9, 125.9, 14.2; MS (ESI⁺): *m/z* [M+H]⁺ Anal. Calcd for C₁₀H₁₁N₂O₃S: 239.0485. Found: 239.0489 (Supplementary Materials).

4.4. MAO Inhibition Studies

The measurement of IC₅₀ values for the inhibition of human MAO-A and MAO-B was carried out according to a previously reported protocol [13]. Recombinant human MAO-A and MAO-B were obtained from Sigma-Aldrich (USA), and fluorescence measurements were recorded 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, was oxidized by the MAOs to yield 4-hydroxyquinoline.

4.5. Molecular Docking Studies

Molecular docking was carried out according to the previously reported protocol using the Discovery Studio 3.1 suite of software [11]. The X-ray crystal structure of MAO-B (PDB code: 2V5Z) complexed to safinamide was used for the docking study [28]. The illustration was created with the PyMOL molecular graphics system [29].

4.6. Cytotoxicity Assay

The human cell culture line HS-5 (bone marrow stroma) was grown in a mixture (1:1) of RPMI-1640 and Ham's F12 media without glutamine. To the media, we added FBS (10%), L-glutamine (2 mM), penicillin (50 IU/mL), and streptomycin (50 µg/mL). The test compounds were dissolved in DMSO and added to the culture media to yield a final concentration of 0.1% DMSO. As a positive control (0% cell viability), the cells were exposed to sodium azide (0.1%). As a negative control, cell viability was measured in the absence of the test compound. The cytotoxicity of the test compound was determined by

the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] protocol [27]. Absorbance was measured at a wavelength of 590 nm with a CLARIOstar (BMG Labtech) spectrophotometer. The blank consisted of culture medium.

Supplementary Materials: ¹H- and ¹³C-NMR spectra copies of synthesized compounds can be found in the File S1 (Supplementary Materials).

Author Contributions: Conceptualization of the study was conducted by A.A.S.; formal analysis and investigation by J.A.E. and A.P.; writing—original draft preparation was conducted by A.A.S.; writing—review and editing was conducted by M.K.K. and J.P.P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no competing interests.

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