SYNTHESIS AND CHARACTERIZATION OF 2-[3-(4-ARYL)-1,2,4-OXADIAZOL-5-YL] BENZOIC ACID DERIVATIVES AS POTENTIAL BIOACTIVE COMPOUNDS

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Introduction

Heterocyclic compounds represent a cornerstone of medicinal chemistry, offering diverse chemical scaffolds that contribute to the development of bioactive molecules, and, for this reason, they continue to yield new therapeutic agents [Kabir and Uzzaman 2022]. The biological activity exhibited by the heterocycles is due to their potential to bind with various enzymes either to the active sites or with enzyme pocket structures through a broad range of intra-molecular interactions such as van der Waals and hydrophobic forces, hydrogen bonding, and metallic coordination bonds, making them an important framework in medicinal chemistry [Boström *et al.* 2012]. Heterocyclic compounds containing nitrogen and oxygen heteroatoms are the most important class of compounds in the pharmaceutical and agrochemical industries, in which heterocycles comprise around 60% of the drug substances. Among these, oxadiazoles have garnered significant interest due to their versatile structural and electronic properties [Nobeli *et al.* 1997].

Oxadiazoles exhibit four isomeric forms: 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,3,4-oxadiazole, and 1,2,5-oxadiazole, each differing in the positioning of nitrogen and oxygen atoms within the aromatic five-membered ring (Figure 1).

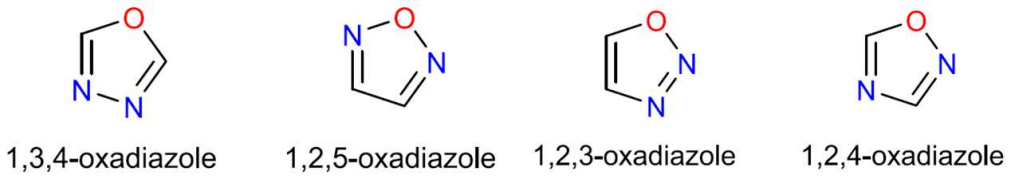


Figure 1. The four isomeric forms of oxadiazole heterocycle.

The 1,2,4-oxadiazole ring has been used to replace amide and ester functionalities [Luthman *et al.* 1999], and plays a central role in producing potent, metabolically stable and bioavailable compounds in many research programs [Baykov *et al.* 2023]. Consequently, 1,2,4-oxadiazole derivatives have been extensively explored for their potential pharmacological applications, including antimicrobial, anticancer, anti-inflammatory, antihypertensive, antidiabetic, antithrombotic, immunomodulatory, neuroprotective and several other activities [Biernacki *et al.* 2020; Khasawneh *et al.* 2025], as depicted in Figure 2.

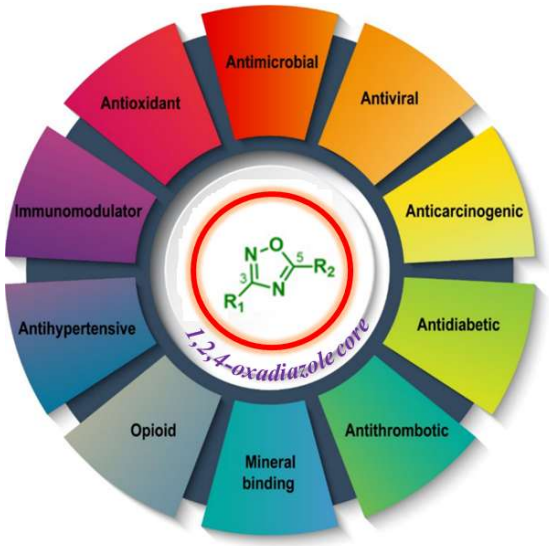


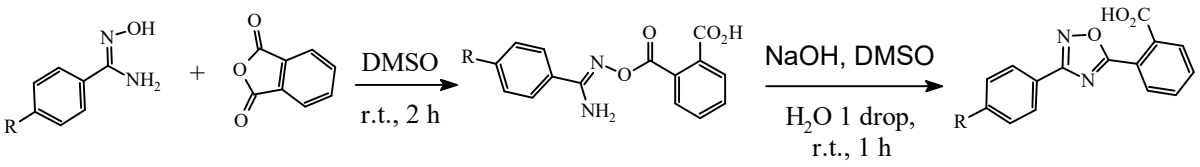
Figure 2. Biological activities associated to the 1,2,4-oxadiazole core.

There are several synthetic strategies developed for synthesizing 1,2,4-oxadiazole derivatives, especially involving the use of amidoxime with carbonyl compounds such as carboxylic acids, aldehydes, 2-chloro-2-oxoacetate, and esters [Dhameliya *et al.* 2022]. Carboxylic acid-bearing 1,2,4-oxadiazole derivatives are promising structures for investigating novel bioactive products, as demonstrated by the commercial drug Ataluren, used in the treatment of cystic fibrosis [Bora *et al.* 2014], as well as starting material for designing more complex molecules. The best synthetic strategy for getting access to 2-[3-(4-aryl)-1,2,4-oxadiazol-5-yl] benzoic acid derivatives is the condensation of amidoximes with dicarboxylic acid anhydrides, leading to O-benzoyl benzamidoximes, which can undergo cyclodehydration under mild conditions in the presence of alkali hydroxides, giving the 1,2,4-oxadiazole ring, whose experimental conditions were previously established at **SintMed®**. The synthetic route for the series 2-[3-(4-aryl)-1,2,4-oxadiazol-5-yl] benzoic acid **4a-g** is given in Scheme 1. Therefore, this work aims, in an early stage, to select several substituents to evaluate the scope of the methofology and their effect on the reaction's efficiency, along with the obtention of a library of potential bioactive molecules.

Material and Methods

Reactions' progress was monitored by thin-layer chromatography (TLC), performed onto glass-backed plates of silica gel 60 PF254 with gypsum from Merck, using AcOEt/AcOH 10 mL:1 drop as eluent, and all compounds were detected by ultraviolet light (254 nm) and iodine. Melting points were determined with a capillary apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz or 500 MHz for hydrogen (^{1}H NMR) and carbon (^{13}C NMR). Analyses were determined at 25 °C in DMSO-d_6 with chemical shift values (δ) in parts per million (ppm).

The synthetic strategy has started by reacting 4-aryl amidoximes **1a-g** and phtalic anhydride **2**, leading to the intermediate O-benzoyl benzamidoximes **3a-g** in DMSO at room temperature. In a "two steps, one-pot" reaction, pulverized NaOH was poured into the reaction mixture and stirred at room temperature until TLC analysis showed the reaction's end. After dilution with an acid solution, the target products 2-[3-(4-aryl)-1,2,4-oxadiazol-5-yl] benzoic acids **4a-g** were obtained as solid powders (Scheme 1).



1a-g **2** **3a-g** **4a-g**

R = H (a), CH_3 (b), F (c), Cl (d), Br (e), NO_2 (f), OCH_3 (g)

Scheme 1. Synthetic route for 2-[3-(4-aryl)-1,2,4-oxadiazol-5-yl] benzoic acids **4a-g**.

Preparation of 2-[3-(4-aryl)-1,2,4-oxadiazol-5-yl] benzoic acids 4a-g: A solution of appropriate 4-aryl amidoxime **1a-g** (0.5 mmol) and phtalic anhydride **2** (0.5 mmol) in 2.0 mL of dimethyl sulfoxide (DMSO) was stirred at room temperature (r.t.) for 2.0 hours, when thin layer chromatography (TLC) indicated the total consumption of both reactants. After this time, the heterocyclization step has been carried out in presence of NaOH (2.0 mol equivalents) and 1 drop water at r.t. for 1.0 hour. TLC control (AcOEt/AcOH 10 mL:1 drop) was used to observe the consumption of both reactants. The reaction mixture was then diluted with a solution of 1.0 mL conc. HCl and 15 mL water to pH ~1, resulting in a precipitate that was filtered off, washed with cold water and dried under vacuum. Results are displayed in Table 1.

Results and Discussion

The previously studied methodology for the synthesis of 2-[3-(4-aryl)-1,2,4-oxadiazol-5-yl] benzoic acids **4a-g** was succesfully applied, and seven pure, novel compounds were obtained as white solids. Yields range from excellent to good and purity of crude compounds was high, avoiding further purification, according to the data in Table 1.

Table 1. Characterization data for the derivatives 2-[3-(4-aryl)-1,2,4-oxadiazol-5-yl] benzoic acids **4a-g**.

Comp.	X	Yield (%)	M.p. (°C)	^{1}H NMR
4a	H	97	157-158	8.09 (d, 2H, Ar), 7.99-7.96 (m, 1H, Ar), 7.95-7.91 (m, 1H, Ar), 7.81-7.77 (m, 2H, Ar), 7.61-7.57 (m, 3H, Ar)
4b	CH_3	86	192-192	7.97-7.94 (m, 3H, Ar), 7.92-7.90 (m, 1H, Ar), 7.80-7.77 (m, 2H, Ar), 7.40 (d, 2H, Ar), 2.39 (s, 3H, CH_3)
4c	F	88	190-191	8.11-8.06 (m, 2H, Ar), 7.95-7.93 (m, 1H, Ar), 7.88-7.87 (m, 1H, Ar), 7.79-7.75 (m, 2H, Ar), 7.40 (t, 2H, Ar)
4d	Cl	88	182-183	8.08 (d, 2H, Ar), 7.98-7.96 (m, 1H, Ar), 7.92-7.90 (m, 1H, Ar), 7.80-7.79 (m, 2H, Ar), 7.67 (d, 2H, Ar)
4e	Br	86	177-178	8.01 (d, 2H, Ar), 7.99-7.97 (m, 1H, Ar), 7.93-7.91 (m, 1H, Ar), 7.82-7.79 (m, 4H, Ar)
4f	NO_2	86	221-222	8.43 (d, 2H, Ar), 8.33 (d, 2H, Ar), 8.04-7.99 (m, 1H, Ar), 7.95-7.93 (m, 1H, Ar), 7.85-7.79 (m, 2H)
4g	OCH_3	84	161-162	7.99-7.94 (m, 1H), 7.93-7.88 (m, 1H), 7.83-7.76 (m, 2H), 7.14 (d, 2H), 3.84 (s, 3H)

The structural features of compounds **4a-g** led to a simple set of signals at ^{1}H NMR analyses. Aromatic rings were clearly characterized for all compounds from δ 8.11 to 7.14 ppm, confirming the cyclization process for all samples and the aromatic rings formation. Except for compounds **4b** and **4g**, which bear a CH_3 group, no other signals were observed at high field. As expected, the signal for carboxylic group couldn't be ascertained due to the proton exchange with the deuterated solvent. However, a ^{13}C NMR (100 MHz, DMSO) for compound **4a** was recorded, giving δ 176.06 (C=O), 167.92 (Ar), 167.37 (Ar), 132.90 (Ar), 132.52 (Ar), 131.97 (Ar), 131.74 (Ar), 130.48 (Ar), 129.86 (Ar), 129.37 (Ar), 127.13 (Ar), 126.15 (Ar), 123.79 (Ar),

in agreement with the proposed structure. Therefore, this result reinforced the ^{1}H NMR data as structural pattern for the entire series.

Conclusions

An efficient, convenient and mild methodology for the synthesis of 2-(3-aryl-1,2,4-oxadiazol-5-yl) benzoic acids **4a-g** was described in a "two steps, one pot" process, leading to 7 novel compounds, substrates for building up more complex molecules, which can be investigated as potential bioactive compounds. The reaction's scope seemed to be very promising, accepting either electron donating or withdrawing groups, encouraging further investigations, looking towards a plethora of substituted compounds.

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