STUDIES ON THE SYNTHESIS OF 2-(3-PHENYL-1,2,4-OXADIAZOL-5-YL) BENZOIC ACID

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Introduction

The use of heterocyclic moieties in the development of new drugs has increased in recent years. In nature, heterocyclic compounds are ubiquitous, giving rise to thousands of bioactive natural products. Among the most important heterocyclic groups found in medicinal chemistry are the oxadiazoles, which bear one oxygen and two nitrogen atoms in a five membered ring. Oxadiazoles possess hydrogen bond acceptor properties [BOSTRÖM *et al.* 2012], owing to electronegativities of nitrogen and oxygen, wherein nitrogen is stronger hydrogen bond acceptor than oxygen [NOBELI *et al.* 1997]. Additionally, oxadiazoles have been recognized as bioisosters of hydroxamic esters, carboxamides and carbamates, leading to metabolic stability to parent scaffolds [PATANI *et al.* 1996]. The aromatic oxadiazole ring has four isomeric forms: 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, and 1,3,4-oxadiazole. The 1,2,4-oxadiazole is a heterocycle, which has been used to replace amide and ester functionalities [LUTHMAN *et al.* 1999], and has central role in producing potent, metabolically stable and bioavailable compounds in many research programs [BAYKOV *et al.* 2023]. A plethora of studies exploit the 1,2,4-oxadiazole derivatives as anticancer, antiparasitic, antifungal, antibacterial, antidepressant, antitubercular and anti-inflammatory novel drugs, among others [BIERNACKI *et al.* 2020]. From these efforts, a few commercially available drugs containing 1,2,4-oxadiazole nucleus such as pleconaril, oxolamine, prenoxdiazine, butalamine, ataluren and proxazole were discovered and put in market [DHAMELIYA *et al.* 2022] (Figure 1).

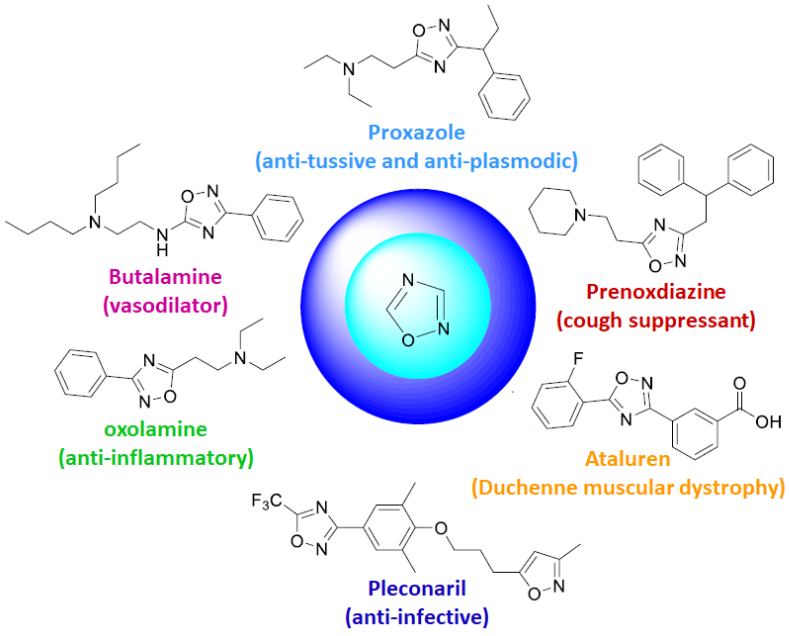


Figure 1. Chemical structures of commercial drugs based on 1,2,4-oxadiazole scaffold.

The 1,2,4-oxadiazole heterocycle was synthesized for the very first time in 1884 by Tiemann and Krüger and was originally classified as azoxime or furo[ab1] diazole, and during more than 100 years several synthetic strategies has been developed, involving the use of amidoxime with carbonyl compounds such as carboxylic acids (Route A), aldehydes (Route B), 2-chloro-2-oxoacetate (Route C), and ester (Route D). Along with these, other routes such as intramolecular cyclization of ester clubbed with amidoxime (Route E) and aminonitrone, hydroxylamine and isocyanide (Route F) (Figure 2) are usefull to yield 1,2,4-oxadiazole rings [DHAMELIYA *et al.* 2022].

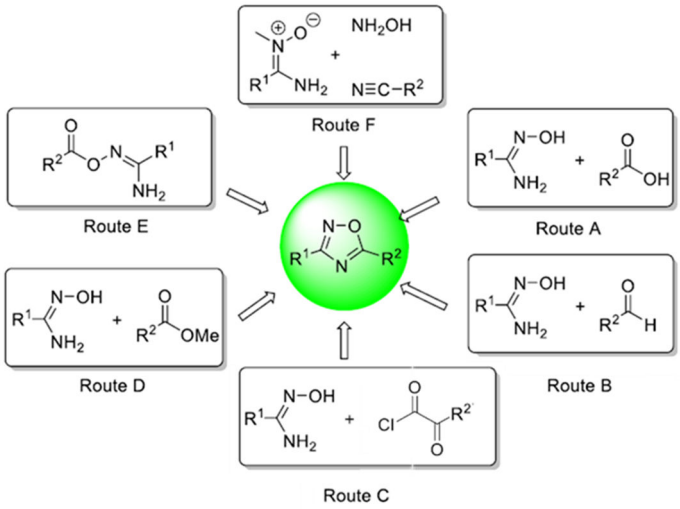


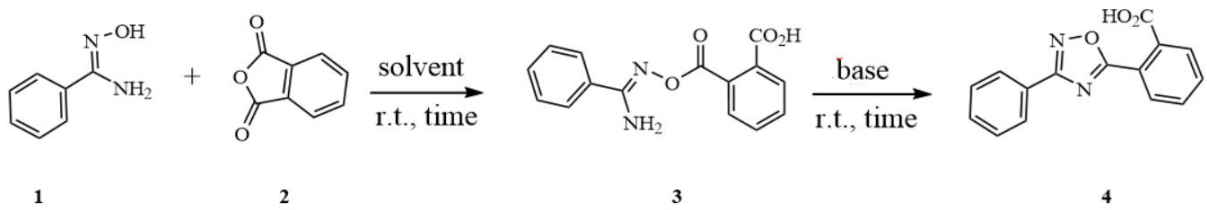
Figure 2. Synthetic strategies for accessing 1,2,4-oxadiazole derivatives.

A less applied methodology for the synthesis of 1,2,4-oxadiazole is the condensation of amidoximes with dicarboxylic acid anhydrides, leading to 1,2,4-oxadiazole-bearing carboxylic acids, which are important substrates for synthesizing more complex molecules. Typically, this reaction is carried out in ‘‘two-step, one-pot” fashion *via* O-acylamidoxime intermediate generation and subsequent thermal cyclodehydration at high temperatures (~100-140 ºC), which often results in poor product yields and the formation of undesired by-products [GODOVIKOVA *et al.* 2008]. However, better results can be reached using basic reagents for the room-temperature synthesis of 1,2,4-oxadiazole derivatives [BAYKOV *et al.* 2016]. Based on these results, our group decided to investigate the ideal conditions for the synthesis 2-(3-phenyl-1,2,4-oxadiazol-5-yl) benzoic acid, in a model synthesis, starting from benzamidoxime and phtalic anhydride under different conditions (Scheme 1).

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, a mixture of 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. IR spectra were recorded on a Tensor27 FTIR spectrometer from Bruker with the samples being analyzed as KBr pellets.

The synthetic strategy has started by investigating the two main experimental parameters for the planned synthesis The first one is the definition of the ideal solvent for the two steps of the synthesis, based on the formation of the intermediate O-benzoyl benzamidoxime **(Product 3)** from benzamidoxime **(Product 1)** and phtalic anhydride **(Product 2)**. The second one is the determination of the better base for the heterocyclization, leading to the target compound 2-(3-phenyl-1,2,4-oxadiazol-5-yl) benzoic acid **(Product 4)** (Scheme 1).



1 2 3 4

Scheme 1. Determination of the ideal conditions for the synthesis of 2-(3-phenyl-1,2,4-oxadiazol-5-yl) benzoic acid.

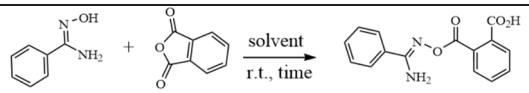
Preparation of the intermediate O-benzoyl benzamidoxime (Product 3): A solution of benzamidoxime **(Product 1)** (0.5 mmol) and phtalic anhydride **(Product 2)** (0.5 mmol) in 2.0 mL of test solvent has been stirred at room temperature (r.t.) until thin layer chromatography (TLC) indicated the total consumption of both reactants. The experiments have been run using THF, DMSO, DMF, water, MeOH, EtOH, ethyl acetate, dichloromethane and toluene. TLC control (AcOEt/AcOH 10 mL:1 drop) has been taken in intervals of 30 minutes during 5.0 hours, and results are given in Table 1.

Preparation of 2-(3-phenyl-1,2,4-oxadiazol-5-yl) benzoic acid (Product 4): A solution of benzamidoxime **(Product 1)** (0.5 mmol) and phtalic anhydride **(Product 2)** (0.5 mmol) in 2.0 mL of appropriate solvent has been stirred at room temperature (r.t.) during the previous established time. After reaction's conclusion, the hetero cyclization has been investigated in presence of different inorganic bases: LiOH, KOH, NaOH, K_2CO_3, Na_2CO_3, Cs_2CO_3, $KHCO_3$ and $NaHCO_3$. TLC control (AcOEt/AcOH 10 mL:1 drop) has been used to observe the consumption of both reactants in intervals of 30 minutes during 5.0 hours. The reaction mixture has been then diluted with a solution of 1.0 mL conc. HCl and 15 mL water to pH ~1. The resulting precipitate has been filtered off, washed with cold water and dried under vacuum. Results have been displayed in Table 2.

Results and Discussion

The formation of O-benzoyl benzamidoxime **(Product 3)** was carried out according to the proposed methodology, and the reaction progression was monitored by TLC (Table 1).

Table 1. Influence of solvents on the formation of O-benzoyl benzamidoxime **(Product 3)**.



1 2 3

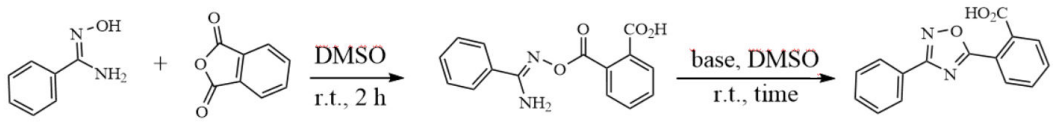
Entry	Solvent	Time (h)	Reactants consumption (TLC observation)
1	THF	5.0	Incomplete
2	DMSO	2.0	Complete
3	DMF	4.5	Complete
4	H_2O	5.0	Not observed
5	MeOH	5.0	Not observed
6	EtOH	5.0	Not observed
7	AcOEt	5.0	Not observed
8	CH_2Cl_2	5.0	Not observed
9	$PhCH_3$	5.0	Not observed

An equimolar mixture of benzamidoxime **(Product 1)** and phtalic anhydride **(Product 2)** was dissolved in the test solvent and stirred at room temperature. Investigations have been started with the aprotic polar solvents, *i.e.*, THF, DMSO and DMF. A partial consumption of both reactants has been observed after 5.0 hours in THF (Entry 1), which is an insufficient result, looking towards a second synthetic step. However, the reactions in DMSO (Entry 2) and DMF (Entry 3) have disclosed excellent results, especially for DMSO, whose TLC control has shown reaction completion in 2 hours.

Interestingly, protic polar solvents (Entries 4-6) have led to complete failure of the formation of the intermediate **3**. The most likely reason for these outcomes is the low solubility of phtalic anhydride in these 3 solvents. Experiments carried out in less polar or apolar solvents (Entries 7-9) have also disclosed no consumption of both reactants. After 5 hours, TLC analyses haven't even shown traces of the expected product. In these cases, it is possible that the reaction doesn't occur due to the polar nature of the intermediates formed during the process, which cannot be stabilized under such conditions. Bottomline, the best solvent for the synthesis of O-benzoyl benzamidoxime **(Product 3)** is DMSO.

Once DMSO has been established as the best solvent for the first reaction step, a series of experiments has been designed to investigate which base would be the most suitable for the heterocyclization reaction. In order to make sure the effectiveness of each base, a set of three repetitions has been carried out. All experiments have been monitored by TLC and isolated yield. Since the intermediate **3** bears a carboxylic acid group, it has been used 2 mol equivalents of each base in the experiments. The overall results are shown in Table 2.

Table 2. Influence of bases on 1,2,4-oxadiazole cyclization.



1 2 3 4

Entry	Base	Equivalent	Time (h)	Isolated yield (%)
1	LiOH	2.0	1.5	95
2	NaOH	2.0	1.0	97
3	KOH	2.0	2.0	97
4	K_2CO_3	2.0	5.0	52
5	Na_2CO_3	2.0	5.0	45
6	Cs_2CO_3	2.0	5.0	43
7	$NaHCO_3$	2.0	5.0	Trace
8	$KHCO_3$	2.0	5.0	Trace
9	NaOH	3.0	2.0	96

The three alkali hydroxides LiOH, NaOH and KOH (Entries 1-3) have afforded the best results for the 1,2,4-oaxadiazole formation, with similar yields in different time reactions. Sodium hydroxide has performed better, but the use of the other two bases is also acceptable. Carbonates K_2CO_3, Na_2CO_3 and Cs_2CO_3 have given lower yields of target product **4** (Entries 4-6) in longer reaction times. The results for the other bases (Entries 7-8) have led to trace amounts of the desired product, which has been identified only by TLC analysis. Thus, alkali metal hydroxides are the most suitable reagents for cyclodehydration under mild conditions. In an attempt to lower the reaction time, an experiment using 3.0 mol equivalent of NaOH has been run (Entry 9), but its outcome has been essentially the same of Entry 2.

During the repetition of experiments disclosed in Entries 1-3, an inconsistent result has been observed for one test with NaOH and other with LiOH. In both cases, lower yields have been recorded for NaOH (55%) and LiOH (62%). Initially, it has been supposed that the hygroscopicity of the bases have affected the results of the reactions. Additional experiments have been run, this time with extreme dry bases. However, instead the expected excellent yields, the results have been even lower. In face of these outcomes, a final experiment has been envisaged. A reaction mixture has been set up, and a drop of water has been poured into it. This time, after 3 runs, all yields observed have been over 95%. Hence, the presence of moisture is important for the 1,2,4-oxadiazole cyclization, probably because it increases the dissolution of the base in DMSO.

Conclusions

Herein, we have described an efficient and convenient method for the synthesis of 2-(3-phenyl-1,2,4-oxadiazol-5-yl) benzoic acid by the cyclodehydration of O-benzoyl amidoxime in the base system MOH/DMSO (M = Na, K, Li). This method uses widely available and inexpensive reagents (DMSO, hydroxides of alkali metals) and provides excellent yields of the desired compound. Besides, it was found out that moisture is necessary for the good progress of the reaction, whose scope is the next investigation step in our research, in order to determine the range of substituents that can be introduced in further experiments.

Acknowledgments

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