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Synthesis and Cytotoxicity Evaluation of 2-Benzoylbenzoxazoles by Reaction of *o*-Aminophenol with Acetophenone Catalyzed by Sulfur in DMSO

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Abstract

The 2-benzoylbenzoxazole derivatives were known for their significant biological activities such as anti-proliferative activity, inhibition of FAAH enzyme (fatty acid amide hydrolase). Synthesis of biologically active 2-benzoylbenzoxazole motifs often relies on a multi-step process or precursors carrying active groups. In this study, we report a simple and efficient method for the synthesis of such compounds by the direct reaction of o-aminophenol with acetophenone catalyzed by sulfur in DMSO. The reaction was found to take place via benzoxazolation in the Willgerodt rearrangement of acetophenone, followed by benzylic oxidation to restore the carbonyl functional group. With the optimized reaction conditions, we have synthesized a number of 2-benzoylbenzoxazoles 3aa - 3ae derivatives with good yield from 60 to 75%. Several synthetic derivatives have shown cancer cell growth inhibitory activity with IC50 values ranging from 36.37 to 56.08 μM. The cytotoxicity of some resulting compounds was evaluated and showed that the ellipticine positive control was stable in the experiment.

Keywords: Benzoylbenzoxazole, Willgerodt, sulfur, DMSO, oxidation

1. Introduction

The 2-benzoylbenzoxazole derivatives were known for their significant biological activities such as anti-proliferative activity, inhibition of FAAH enzyme (fatty acid amide hydrolase). Therefore, there have been several synthesis methods of 2-benzoylbenzoxazole [1,2], most of which rely on reconstructing the basic benzoxazole skeleton [3-5] or using complex starting materials and multi-step synthesis [6].

The synthesis approach for the benzoxazole ring is to condense o-aminophenol 1 with phenylglyoxalic derivatives such as dithioester or imidoyl cyanide via non-redox condensation. In addition, when the oxidizing conditions are required, compound 1 will condense with derivatives having weak oxidizing capacity such as α,α-dihaloacetophenone or 2-bromophenyllacetylene. In this study, the direct use of acetophenone 2, which is inexpensive and readily available with a wide variety of structures, to perform a selective oxidative condensation with o-aminophenol 1 provides an efficient and economical synthesis approach to access compounds containing the 2-benzoylbenzoxazole scaffold with many applications in the pharmaceutical field.

In this study, we investigated the Willgerodt rearrangement and benzoxazolation between acetophenone 2 and o-aminophenol 1 catalyzed by sulfur [6,7] and N-methylpiperidine (Fig. 1).

This rearrangement results in benzoxazole **4**, while the methyl group of acetophenone **2** is oxidized and benzoxazolation with 2-aminophenol **1**, the carbonyl group is reduced to the methylene group. The methylene group located between the phenyl group and the newly formed benzoxazole nucleus **4** is further oxidized to the carbonyl to obtain **3**. This process has been described for 2-benzylbenzoxazole synthesis under relatively complex reaction conditions and using strong oxidizing agents in the presence of transition metal catalysts [7-10]. We aimed to transform directly from **1** and **2** to **3** in an "one-pot" reaction with a suitable oxidizing agent in the reaction medium. Therefore, we have focused on investigating a reaction using DMSO as not only a solvent but also a selective mild oxidizing agent, especially in the presence of sulfur [11-13].

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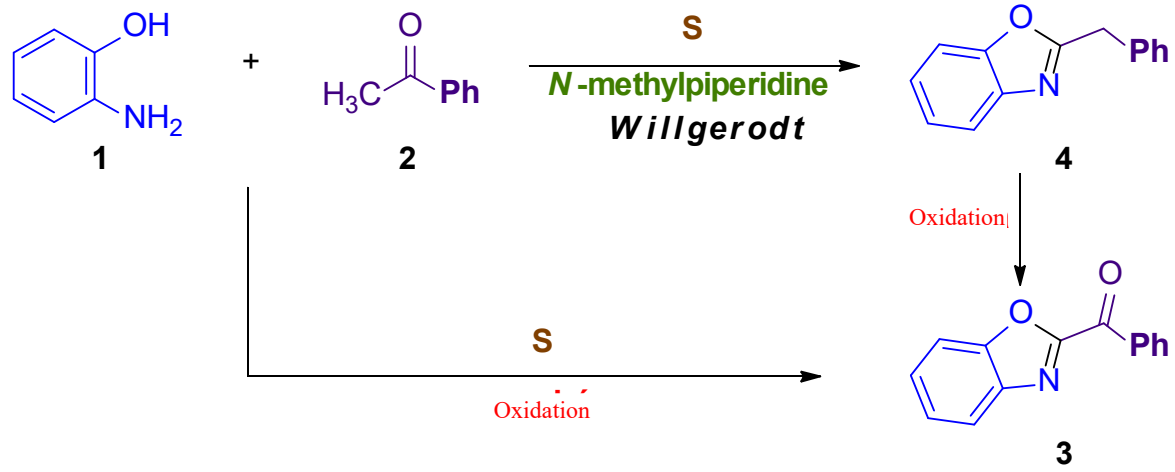


Fig. 1. Synthesis of the benzoxazole ring by the Willgerodt rearrangement between acetophenone **2** with *o*-aminophenol **1** catalyzed by sulfur

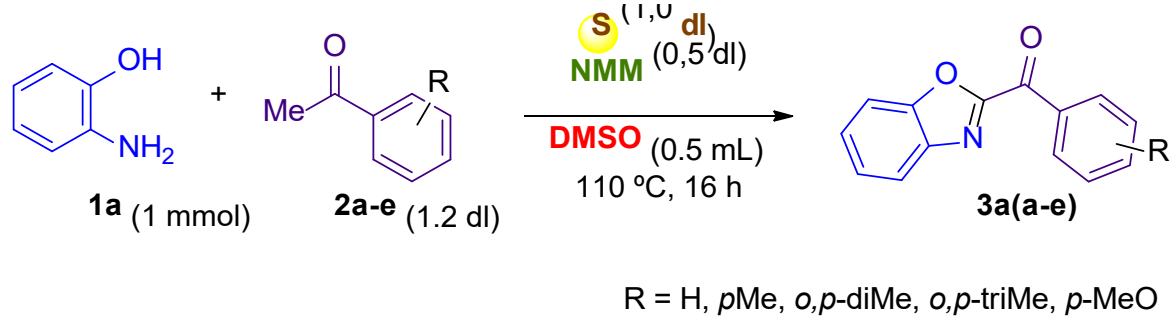


Fig. 2. Synthesis of the benzoxazole by the reaction of *o*-aminophenol and acetophenone catalyzed by sulfur/DMSO system

2. Experiment

The NMR spectra were recorded using a Brucker DRX 500 spectrometer or Varian 400-MR spectrometer. All coupling constants (*J*) were expressed in Hz. The chemical shifts (δ) was expressed in ppm relative to tetramethylsilane, using $CDCl_3$, DMSO as the solvent. The HRMS spectra were obtained using SCIEX-X500R QTOF LC/MS system. Column chromatography was performed using silica-gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) and thin layer chromatography (TLC) was performed using a precoated silica gel 60 F254 (0.25 mm, Merck).

2.1. General Procedure

The mixture of reaction of 2-aminophenol 1 (1 mmol), acetophenone 2 (1.2 mmol), S (32 mg, 1 mmol), N-methylpiperidine (56 mg, 0.5 mmol) and DMSO (0.5 mL) was heated under nitrogen gas atmosphere at 110 °C during 16 hours. The reaction mixture was purified by column chromatography silica gel (heptane: EtOAc 1:0 to 5:1 or dichloromethane: heptane 2:1 to 1:0) (Fig. 2).

2.2. Cytotoxic Assay

Cytotoxic assays were performed according to a method developed by Monks, which is being used at the National Institute of Health (USA) as a standard method for the evaluation of the cytotoxic potential of compounds or extracts using a panel of human cancer cell lines. The cancer cell lines MCF7 (human breast cancer), Hep-G2 (Hepatocellular carcinoma) were provided by Prof. J. M. Pezzuto, Long Island University, US and Prof. Jeanette Maier, University of Milan, Italy and used for the assays.

The cells were cultured as a monolayer in Dulbeco's Modified Eagle Medium (DMEM) or RPMI-1640 (depend on the cell lines) with contents including 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate and supplemented with fetal bovine serum (FBS) 10%. The MCF7 medium was further added with 0.01 mg/ml bovine insulin. The cells were subcultured after 3-5 days with a ratio of 1 : 3 and incubated at 37 °C, 5% CO_2 and 100% humidified. The inhibitory rate of cell growth (IR) of cells was calculated using the following equation:

IR = 100%-[(ODt – OD0) / (ODc - OD0)] x 100, where:

- ODt is average OD value on day 3;

- OD0 is average OD value at time-zero;

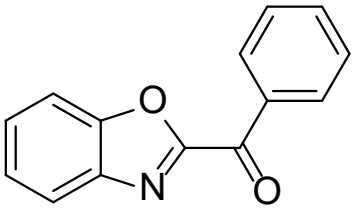
- ODc is average OD value of the blank DMSO control sample.

The cytotoxicities were calculated and expressed as iInhibition concentration at 50% (IC_{50} values). Ellipticine was served as a positive control. In our experiments, the isolated compounds were dissolved in DMSO 100% at 4 mg/ml as stock solution. The final concentration of testing compound for screening assay is 20 µg/ml. The IC_{50} values of promising agents will be determined by testing a series of sample concentrations at 100, 20, 4 and 0.8 µg/ml. Experiments were carried out in triplicate for accuracy of data. The TableCurve 2Dv4 software was used for data analysis and for IC_{50} calculation. The IC_{50} values should be of small deviation throughout the experiments.

3. Results and Discussion

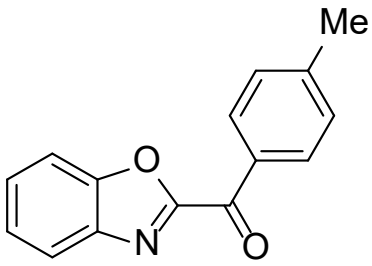
The mixture of reaction of 2-aminophenol 1 (1 mmol), acetophenone (1.2 mmol), S (32 mg, 1 mmol), N-methylpiperidine (56 mg, 0.5 mmol) and DMSO (0.5 mL) was heated under nitrogen gas atmosphere at 110 ºC during 16 hours. The reaction mixture was purified by column chromatography silica gel (heptane: EtOAc 1:0 to 5:1 or dichloromethane:heptane 2:1 to 1:0).

3.1. Benzoxazol-2-yl(phenyl)methanone (3aa, 62%)



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57-8.55 (m, 2H); 7.97-7.95 (m, 1H); 7.73-7.68 (m, 2H); 7.59-7.55 (m, 3H); 7.50-7.47 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.7; 157.3; 150.6; 141.0; 135.2; 134.5; 131.2; 128.9; 128.8; 128.7; 128.6; 125.9; 122.6; 112.1; 112.0 (Fig. 3).

3.2. Benzoxazol-2-yl(p-tolyl)methanone (3ab, 75%)



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48-8.45 (m, 2H); 7.96-7.94 (m, 1H); 7.73-7.70 (m, 1H); 7.57-7.53 (m, 1H); 7.49-7.46 (m, 1H); 7.38-7.36 (d, J = 7.8 Hz, 2H); 2.48 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.2; 157.3; 150.4; 145.5; 140.8; 132.6; 131.2; 129.4; 128.3; 125.7; 122.4; 21.9; 11.9 (Fig. 4).

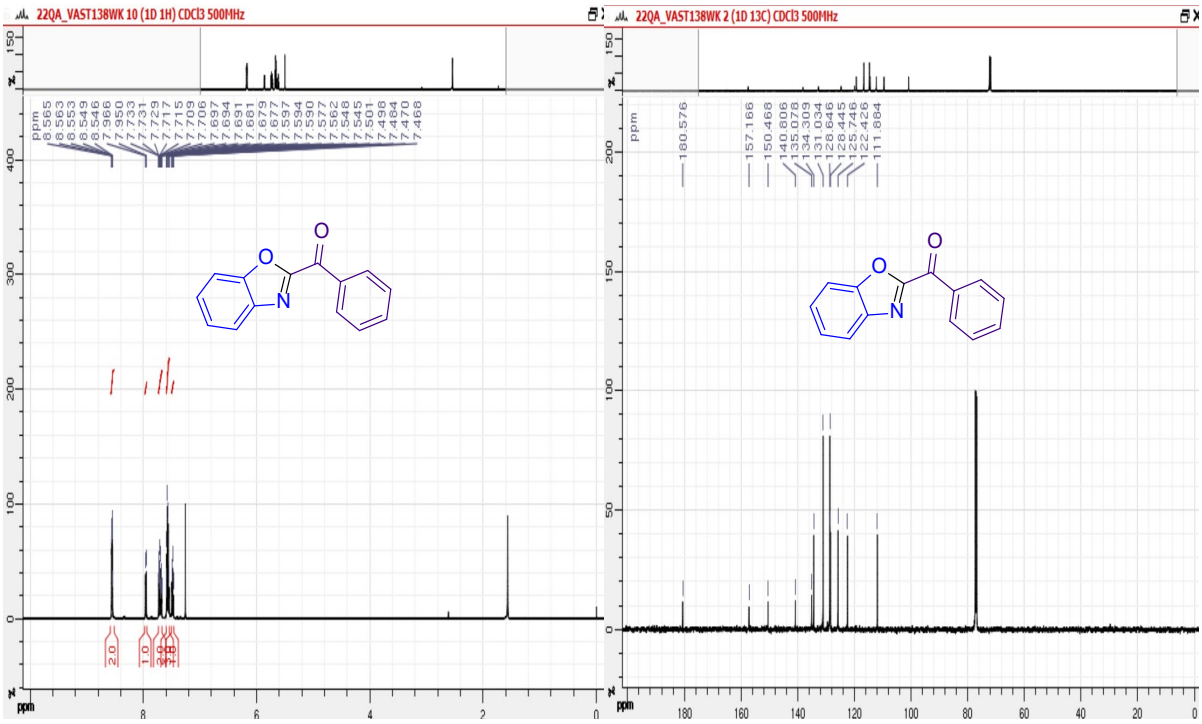


Fig. 3. NMR spectra of Benzoxazol-2-yl(phenyl)methanone **(3aa)**

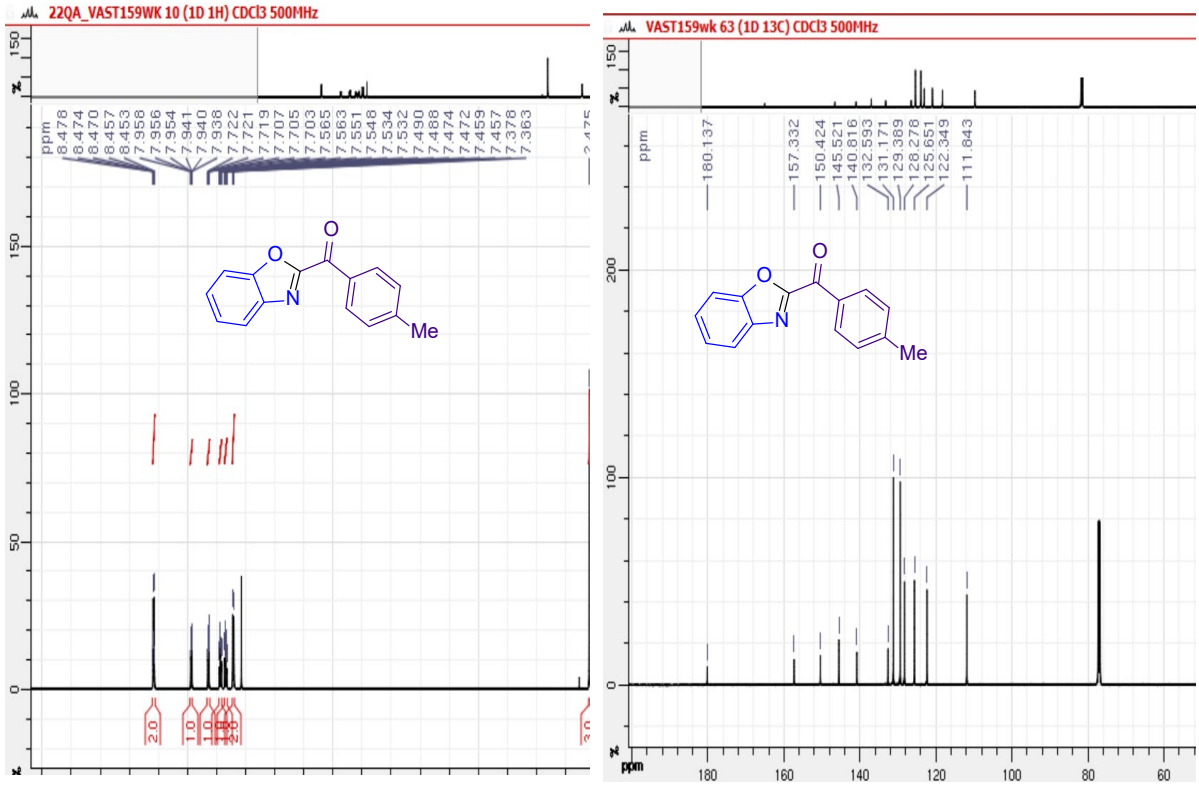


Fig. 4. NMR spectra of benzoxazol-2-yl(p-tolyl)methanone **(3ab)**

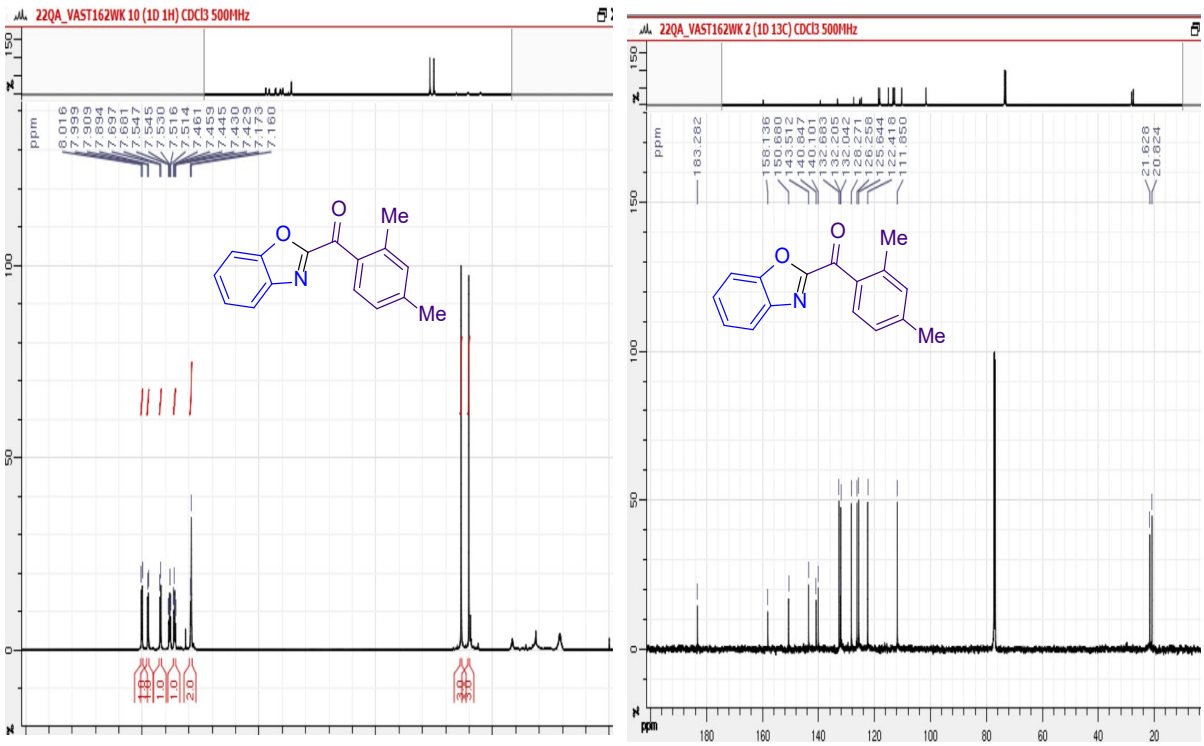
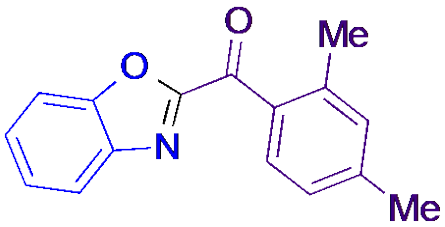


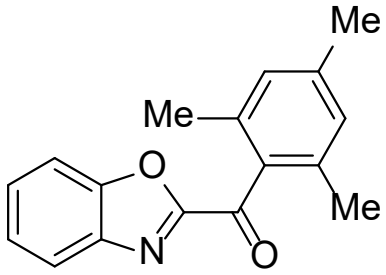
Fig. 5. NMR spectra of 2-(4-methoxyphenyl)benzoxazole **(3ac)**

3.3. 2-(4-Methoxyphenyl)benzoxazole (3ac, 67%)



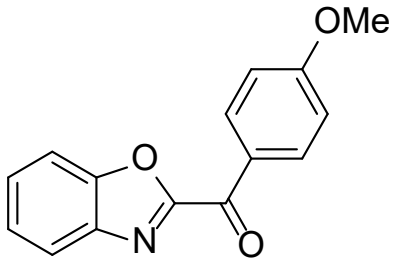
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02-8.00 (d, J = 8.3 Hz, 1H); 7.91-7.90 (d, J = 8.0 Hz, 1H); 7.70-7.68 (d, J = 8.3 Hz, 1H); 7.55-7.52 (m, 1H); 7.46-7.43 (m, 1H); 7.17-7.16 (m, 2H); 2.54 (s, 3H); 2.41 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.3; 158.1; 150.7; 143.5; 140.8; 140.1; 132.7; 132.2; 132.0; 128.3; 126.3; 125.6; 122.4; 111.9; 21.6; 20.8. HRMS (ESI^+) $C_{16}H_{14}NO_2$ $[M+H]^+$ 252.1025; calc 252.1034 (Fig. 5).

3.4. Benzoxazol-2-yl(mesityl)methanone (3ad, 71%)



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89-7.88 (d, J = 8.1, 1H); 7.70-7.68 (d, J = 8.1, 1H); 7.57-7.53 (m, 1H); 7.46-7.43 (m, 1H); 6.95 (s, 2H); 2.35 (s, 3H); 2.21 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 188.5; 158.1; 150.9; 141.0; 140.4; 135.3; 134.7; 128.9; 128.7; 125.8; 122.8; 112.0; 21.3; 19.6. HRMS (ESI^+) $C_{17}H_{16}NO_2$ $[M+H]^+$ 266.1181; calc 266.1194 (Fig. 6).

3.5. Benzoxazol-2-yl(4-methoxyphenyl)methanone (3ae, 62%)



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63-8.60 (m, 2H); 7.95-7.93 (m, 1H); 7.72-7.70 (m, 1H); 7.56-7.52 (m, 1H); 7.49-7.45 (m, 1H); 7.06-7.03 (m, 2H); 3.93 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.8; 164.7; 157.5; 150.4; 140.8; 133.6; 128.1; 128.1; 125.6; 122.2; 114.0; 111.8; 55.6 (Fig. 7).

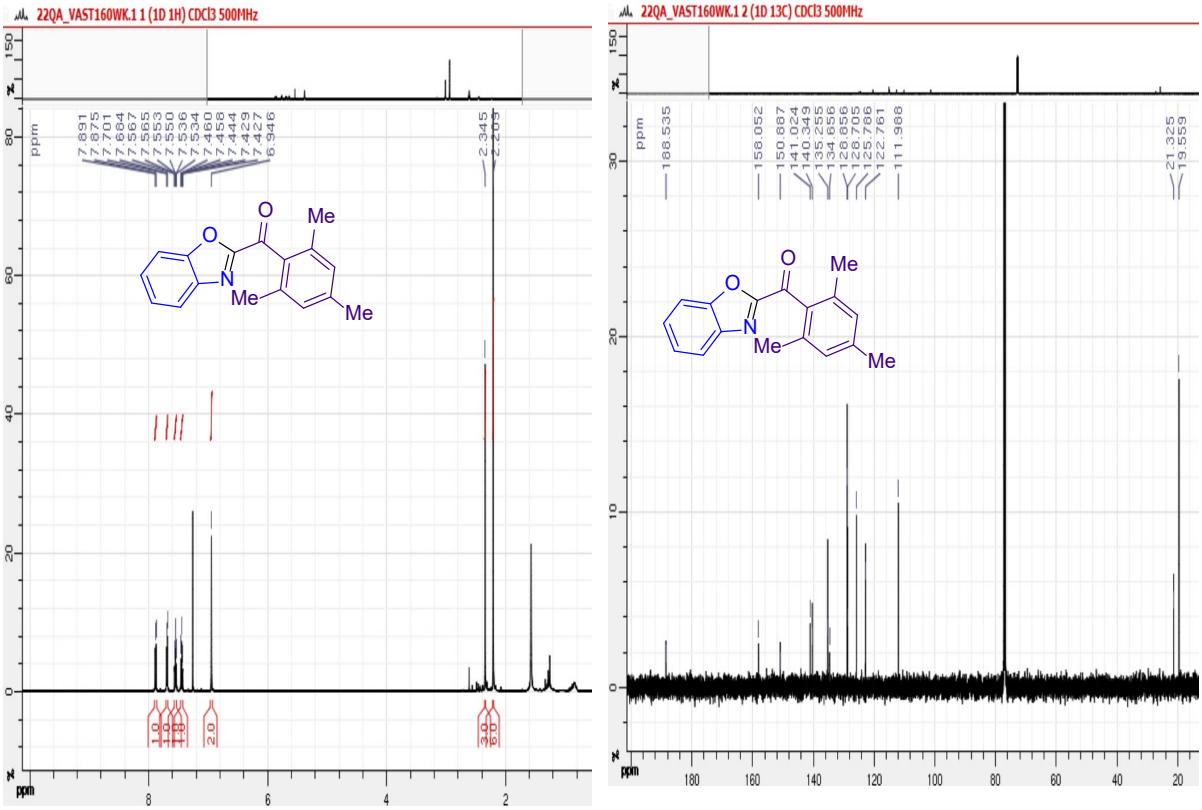


Fig. 6. NMR spectra of Benzoxazol-2-yl(mesityl)methanone **(3ad)**

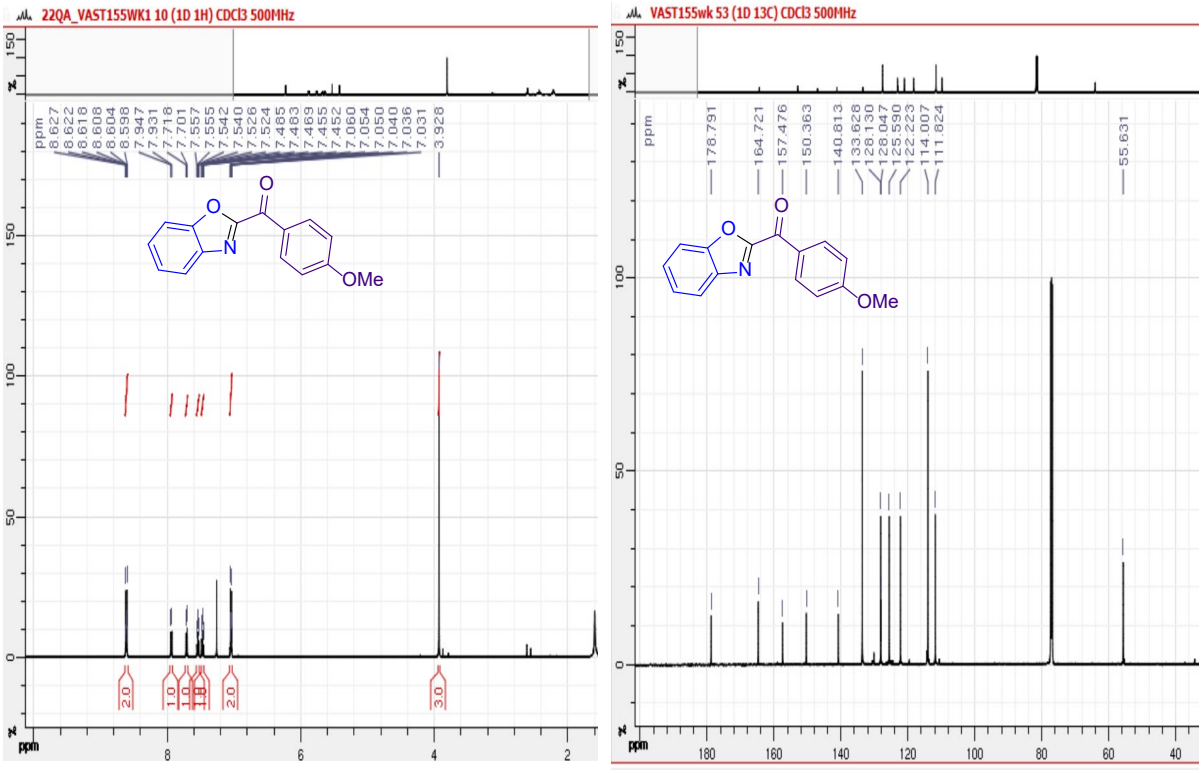


Fig. 7. NMR spectra of Benzoxazol-2-yl(4-methoxyphenyl)methanone **(3ae)**

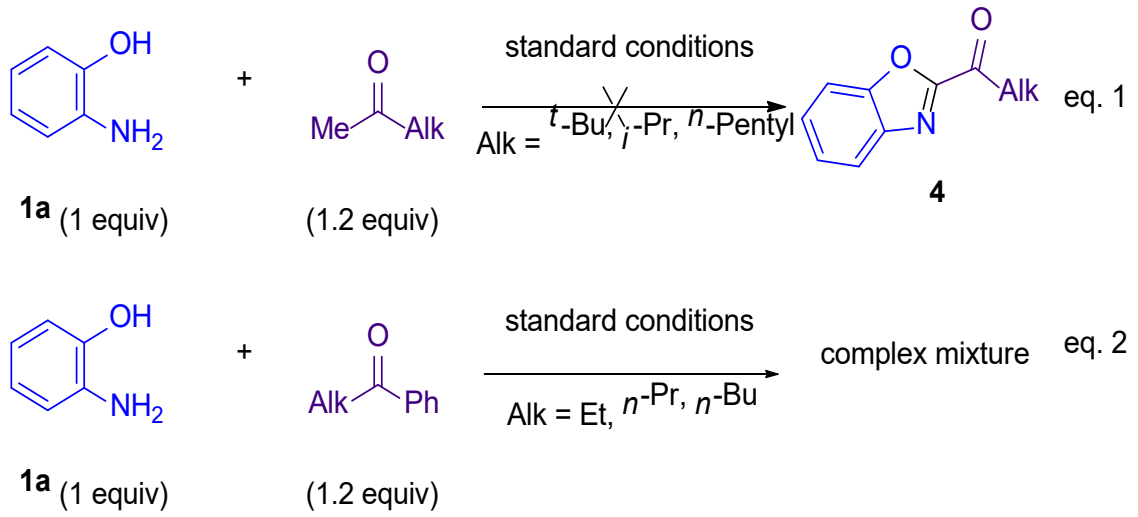


Fig. 8. Screening of reaction scope

Under the conditions of 80 °C, 16 hours, S (3 eq), *N*-methylpiperidine (NMP, 1 eq) and DMSO (3 eq), this initial reaction consumed 80% 2-aminophenol **1** and obtained a mixture of compound **4** and **3** with ratio 1:1. The oxidation of methylene was greatly increased by increasing the reaction temperature (110 °C). At this point, we found that DMSO could act as an oxidant to regenerate sulfur from the H_2S by- product from the first step of Willgerodt-type benzoxazolation. The reaction in the absence of sulfur leads to no product, which clearly confirms the important role of this element in promoting the reaction. Finally, reducing the amount of DMSO or replacing DMSO with a less polar solvent such as DMF partially or completely reduced the formation of **3**, which confirms the importance of DMSO in this oxidative condensation. Under optimized reaction conditions (Fig. 2), we have synthesized a number of 2-benzoylbenzoxazole **3aa-3ae** derivatives with good yield from 60 to 75%.

Table 1. The cytotoxic activities of the studied compounds

Compounds	IC_{50} (µM)			
	LU-1	HepG-2	MCF-7	HT29
3aa	>100	>100	>100	>100
3ac	46.82 ± 1.88	48.68 ± 3.96	36.37 ± 4.42	40.31 ± 2.33
3ad	49.09 ± 1.89	38.47 ± 2.22	56.09 ± 2.14	44.11 ± 1.40
ellipticine	0.34 ± 0.04	0.33 ± 0.03	0.41 ± 0.05	0.52 ± 0.05

However, the reaction conditions are not applicable to unsaturated aliphatic methyl ketones such as pinacolone, 3-methyl-2-butanone and 2-heptanone (Fig. 8, Equation 1). In these cases, the reaction product mixture is quite complex, possibly because both the benzoxazole step of methyl ketones and the oxidation of methylene are more difficult in the absence of the aryl group. Indeed, the products produced from the first step may be in the crude mixture, but subsequent methylene radical oxidation is unsuccessful. The reaction conditions applied to acetophenone homologues such as propiophenone, butyrophenone or valerophenone yield complex mixtures because the Willgerodt reaction intermediates can be oxidized by DMSO in an undesirable manner (Fig. 8, Equation 2)

We evaluated the cytotoxicity of some resulting compounds (Table 1). The obtained results showed that compounds **3ac**, **3ad** showed inhibitory activity with IC_{50} values ranging from 36.37 - 56.08 µM. Compound **3aa** did not have any activity at the concentrations studied. The ellipticine positive control was stable in the experiment. The cytotoxicity results screened *in vitro* on some experimental cancer cell lines, controlled with the standard ellipticine, are valuable to guide further extensive studies on the design, synthesis and investigation of anti-proliferative mechanism.

4. Conlusion

In conclusion, we report an efficient and economical method for the one-pot synthesis of 2-benzoylbenzoxazoles from 2-aminophenol and acetophenone via sulfur-catalyzed Willgerodt rearrangement benzoxazolation and methylene oxidation in DMSO. This method is distinguished by the fact that both *o*-aminophenol and acetophenone starting materials are inexpensive and readily available and structurally diverse. Furthermore, since sulfur and DMSO are used as oxidizing agents with basic catalysts such as N-methylmorpholine, the method is quite simple and cost-effective to prepare 2-benzoylbenzoxazole compounds.

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