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Synthesis and acute toxicity evaluation of Hantzsch 1,4-dihydropyridine derivatives

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ABSTRACT

The derivatives of 1,4-dihydropyridines are well known as calcium channel modulators for the treatment of cardiovascular disorders. Herein, three 1,4-dihydropyridines were synthesized through the condensation of an aldehyde, a β-ketoester, and ammonium acetate in ethanol with yields ranging from 23 to 59%. Their structures were confirmed by comparison HRMS, NMR spectral data with the literature. The acute oral toxicity study for the synthesized compounds revealed that all compounds were safe up to 2,000 mg/kg, and no deaths of animals were recorded.

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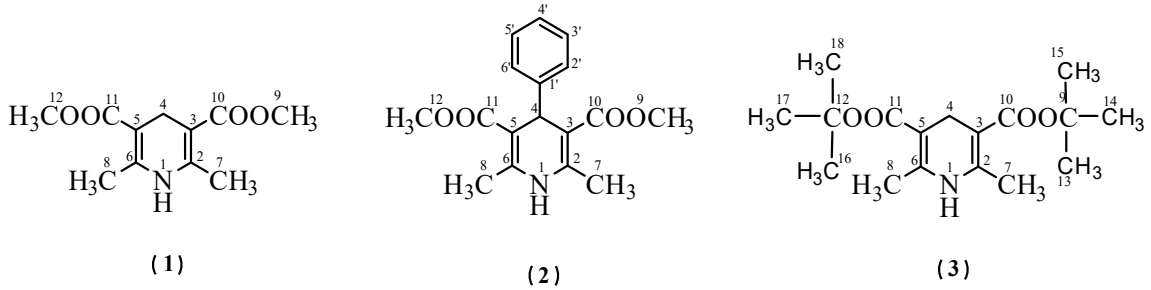
1 INTRODUCTION

Described more than one century ago by Hantzsch, 1,4-dihydropyridines (1,4-DHPs) have now been recognized as effective drugs for the treatment of angina and hypertension (Eynde and Mayence, 2003). Some 1,4-DHPs drugs are commercialized as Amlodipine, Felodipine, Isradipine, Lacidipine, Nicardipine, Nifedipine, Nimodipine and Nitrendipine. Due to their capacity to bind to calcium channels and consequently decrease the transmembrane calcium current, associated in smooth muscle, their effects result in long-lasting relaxation and in a reduction of contractility throughout the heart (Love *et al.*,1974; Bossert *et al.*, 1981). The original Hantzsch dihydropyridine synthesis consists of a multicomponent reaction of acetoacetate esters with aldehyde-ammonia (Hantzsch, 1881). To perform this condensation, the reaction mixture is usually refluxed for several hours (> 10 hrs.). Herein, a simple 1,4-DHPs synthetic method with improving reaction time was reported. The acute toxicity of the three synthesized 1,4-DHPs (Figure 1) was then evaluated. The results provided important information in search for potential pharmacologically active drugs.



(1) (2) (3)

Fig. 1: Structures of the synthesized 1,4-dihydropyridines

2 EXPERIMENTS

2.1 General experimental procedures

The NMR experiments were performed on a Bruker DMX 300 spectrometer. Proton chemical shifts were reported in parts per million (ppm) from tetramethylsilane (TMS). ESI-HRMS was carried out on a MICROMASS ZabspecTOF spectrometer for electrospray ionization. Melting point was recorded on a Krüss Melting Point Meters M5000. Thin layer chromatography was performed on Kieselgel 60F_{254} plates (Merck), and spots were visualized under UV light or sprayed with vanillin (0.5 g vanillin in 80 mL sulfuric acid and 20 mL ethanol), then heated. All solvents used were purchased from Chemsol, purity ≥ 99.0 %.

2.2 Synthesis of 1,4-dihydropyridine derivatives

The three synthesized 1,4-DHPs were performed through Hantzsch reaction (Hantzsch, 1881) with some detailed modifications.

Dimethyl 2,6-dimethyl-1,4-dihydropyridin-3,5-dicarboxylate (1): a mixture of hexamethylenetetramine (560 mg, 5 mmol), methyl acetoacetate (1,160 mg, 10 mmol), ammonium acetate (539 mg, 7 mmol) and ethanol (1 mL) was heated in a 100 mL flask at 80°C for 20 minutes with magnetic stirrer.

Dimethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridin-3,5-dicarboxylate (2): a mixture of benzaldehyde (530 mg, 5 mmol), methyl acetoacetate (1,160 mg, 10 mmol), ammonium acetate (539 mg, 7 mmol) and ethanol (1 mL) was heated in a 100 mL flask at 80°C for 20 minutes with magnetic stirrer.

Di-*tert*-butyl 2,6-dimethyl-1,4-dihydropyridin-3,5-dicarboxylate (3): a mixture of hexamethylenetetramine (560 mg, 5 mmol), *tert*-butyl acetoacetate (1,520 mg, 10 mmol), ammonium acetate (539 mg, 7 mmol) and ethanol (1 mL) was heated in a 100 mL flask at 80°C for 20 minutes with magnetic stirrer.

After cooling, the products were precipitated as yellow crystals which were collected by filtration. Recrystallization from ethanol gave pure products.

Compound (**1**): yield 59%; yellow powder; R_f = 0.54 (chloroform:ethyl acetate 85:15); M.p 227°C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta_{H\ ppm}$ 5.20 (*s*, 1H, NH); 3.24 (*m*, 2H, H4); 3.68 (*s*, 6H, OCH_3×2); 2.17 (*s*, 6H, CH_3×2); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta_{C\ ppm}$ 168.6 (C10, C11); 145.3 (C2, C6); 99.5 (C3, C5); 51.2 (C9, C12); 25.0 (C4); 19.3 (C7, C8). HRMS (ESI$^+$): *m/z* calcd for $C_{11}H_{16}NO_4$ $[M+H]^+$ 225.1079; found 225.1085.

Compound (**2**): yield 23%; white powder; R_f = 0.64 (chloroform:ethyl acetate 85:15); M.p 199°C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta_{H\ ppm}$ 7.23 (*m*, 5H, H2′-H6′); 5.80 (*m*, 1H, H4); 5.03 (*s*, 1H, NH); 3.66 (*s*, 6H, OCH_3×2), 2.35 (*s*, 6H, CH_3×2); ^{13}C NMR (75MHz, $CDCl_3$): $\delta_{C\ ppm}$ 168.2 (C10, C11); 147.5 (C1′); 144.4 (C2, C6); 128.1 (C3′, C5′); 127.7 (C2′, C6′); 126.3 (C4′); 103.9 (C3, C5); 51.1 (C9, C12); 39.4 (C4); 19.7 (C7, C8). HRMS (ESI$^+$): *m/z* calcd for $C_{17}H_{19}NO_4$ $[M+H]^+$ 301.1314; found 301.1327.

Compound (**3**): yield 55%; pale yellow powder; R_f = 0.72 (chloroform:ethyl acetate 85:15); M.p 137°C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta_{H\ ppm}$ 5.16 (*s*, 1H, NH), 3.13 (*m*, 2H, H4), 2.11 (*s*, 6H, CH_3×2), 1.44 (*s*, 18H, CH_3×6); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta_{C\ ppm}$ 167.8 (C10, C11); 144.0 (C2, C6); 100.9 (C3, C5); 79.6 (C9, C12); 28.5 (C13-C18); 25.6 (C4); 19.4 (C7, C8). HRMS (ESI$^+$): *m/z* calcd for $C_{17}H_{28}NO_4$ $[M+H]^+$ 310.2018; found 310.2021.

2.3 Acute toxicity study

The acute oral toxicity study for compounds **1**, **2** and **3** was carried out by following the Organisation for Economic Co-operation and Development (OECD) guidelines (Vijesh *et al.*, 2011) and the lethal dose 50 (LD_{50}) was determined by Litchfield-Wilcoxon method (Litchfield *et al.*,1949). The male and female mice weighing 18-22 g were used for the evaluation. The experiments were performed in stable conditions such as the light of 100 ± 5 lux, the temperature of 27 ± 1ºC and the minimized noise. Each group consisted of 6 mice. Different doses from 100 to 2,000 mg/kg (100 mg/kg, 200 mg/kg, 400 mg/kg, 800 mg/kg, 1,000 mg/kg and 2,000 mg/kg) were selected and administered orally as a single dose as fine suspension prepared in double distilled water using Tween 80. The acute toxic symptoms, the behavioral changes and the death percentage of each group produced by the test compounds were observed and recorded continuously after the first 30 minutes, during the first 24 hours, and daily thereafter for a total of 14 experiment days.

3 RESULTS AND DISCUSSION

Multi-component reactions are suitable for green syntheses as three or more components being combined in reaction to form a final product. Belonging to this kind of transformation, Hantzsch reaction has attracted much attention in the synthesis of 1,4-DHPs due to its high atom economy, selectivity and environmental friendliness. The three 1,4-DHPs (**1**, **2** and **3**) were synthesized

with time and energy efficiency as well as with simple purification steps. The pure products were obtained in yield from 23 to 59%. The low yield obtained in the case of **2** could be explained by steric hindrance of aromatic moiety of benzaldehyde group. In this study, hexamethylenetetramine was used as the source of the ammonia-formaldehyde mixture. Ammonium acetate (7 mmol) was added to the reaction medium in order to obtain the stoichiometric balance between ammonia and formaldehyde. Compounds **1** and **3** have been previously reported to be synthesized by multi-component condensation reaction of hexamethylenetetramine, acetoacetate ester, and ammonium acetate in excess of solvent (Uldrikis *et al*., 1975) or under microwave irradiation (Torchy *et al*., 2002) or ultrasonic irradiation with catalyst (Kumar and Maurya, 2008). Here, such compounds were synthesized with similar yields but under mild conditions, short reaction time and solvent quantity decrease (Figure 2). Their structures were elucidated by spectroscopic data and compared with those in references (Koukabi *et al.*, 2012; Ferraro *et al.*, 2016; Zhang *et al.*, 2017).

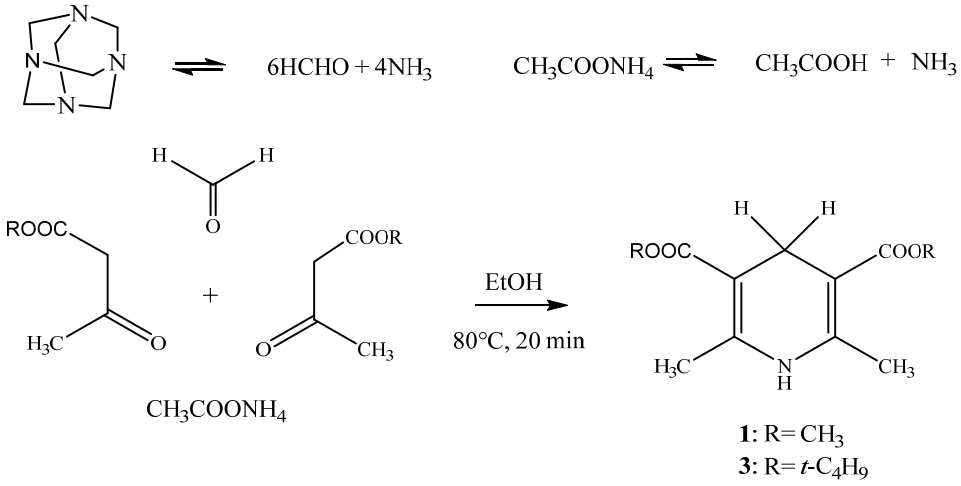


Fig. 2: Synthesis of compounds 1 and 3

The experimental toxicity results showed that no deaths of animals were recorded when orally using the certain increasing doses from 100 mg/kg to 2,000 mg/kg after different time of 30 minutes, 24 hours and up to 72 hours, and the dose of 2,000 mg/kg was the largest dose of each synthesized 1,4-DHP (**1**, **2** and **3**) that could be prepared as the stably oral suspension and orally administered. Moreover, no significant behavioral changes were observed in tested mice during 14 days.

4 CONCLUSIONS

In summary, a rapid and efficient condensation for the synthesis of 1,4-DHPs in moderate to reasonable yields was described. No acute toxicity was observed at the maximum dose of 2,000 mg/kg. The results provide useful information for further pharmacological study on such derivatives for the development of new agents.

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