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Synthesis, crystal structures, fluorescence and xanthine oxidase inhibitory activity of pyrazole-based 1,3,4-oxadiazole derivatives

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ABSTRACT

A series of pyrazole-based 1,3,4-oxadiazole derivatives were rationally designed and synthesized in good yields by following a convenient route. All the newly synthesized molecules were fully characterized by IR, ^{1}H NMR and elemental analysis. Eight compounds were structurally determined by single crystal X-ray diffraction analysis. The fluorescence properties of all the compounds were investigated in dimethyl sulfoxide media. In addition, these newly synthesized compounds were evaluated for *in vitro* inhibitory activity against commercial enzyme xanthine oxidase (XO) by measuring the formation of uric acid from xanthine. Among the compounds synthesized and tested, **3d** and **3e** were found to be moderate inhibitory activity against commercial XO with IC_{50} = 72.4 μM and 75.6 μM. The studies gave a new insight in further optimization of pyrazole-based 1,3,4-oxadiazole derivatives with excellent fluorescence properties and XO inhibitory activity.

1. Introduction

Xanthine oxidase (XO) is a key enzyme that catalyses the oxidation of hypoxanthine and xanthine to generate uric acid in catabolic sequence of the purine nucleotide metabolism in humans and a few other uricotelic species [1]. Overproduction of uric acid levels in serum plays a crucial role in metabolic disorder such hyperuricemia, which further leads to gout and has become a risk of chronic kidney disease and cardiovascular disease [2,3]. In addition, increase in XO serum levels has been involved in various pathological states like hepatitis, mutagenesis, inflammation, cancer and ageing [4–6]. Therefore, the inhibition of XO activity may prove to be a promising therapy to treat gout and other hyperuricemia associated diseases, hepatitis, inflammation, and cancer [7]. Allopurinol, a purine analogue is the first XO inhibitor and has been widely used in clinical management of gout for several decades. However, the adverse effects including allergy, hypersensitivity, syndrome, and renal toxicity caused by the use of allopurinol make it of great importance to develop novel XO inhibitors with minor side effects [8]. In the recent years, several synthetic skeletons containing pyrazoline, pyrimidine, thiazole and triazole have been reported to display XO inhibitory activity [9–14].

1,3,4-Oxadiazole have attracted particular attention due to their fluorescence properties and various biological activities like enzyme inhibition, anti-convulsant, anti-microbial, anti-inflammatory, anti-tumour activities [15–19]. Pyrazole derivatives are well known for their applications in fluorescence probes and pharmacological activities such as anti-microbial, anti-inflammatory, anti-cancer, and antiviral [20–23]. Recently, pyrazoles were found to possess inhibitory activities against XO, cyclooxygenase, and alkaline phosphatases [24,25]. The modification of pyrazole and oxadiazole such as substituent moiety should provide potential fluorescence properties and biological activities. Although much efforts have been put into the synthesis and biological evaluation of pyrazoles and oxadiazoles and numerous corresponding derivatives with fluorescence properties and diverse biological activities including XO inhibitory activity have been reported [26–29], pyrazole-based 1,3,4-oxadiazole sulfur ethers as inhibitors of XO have not been studied. In this work, we reported the facile synthesis, structural analysis, fluorescence and XO inhibitory activity of pyrazole-based 1,3,4-oxadiazole derivatives. The synthetic reactions are summarized in Scheme 1.

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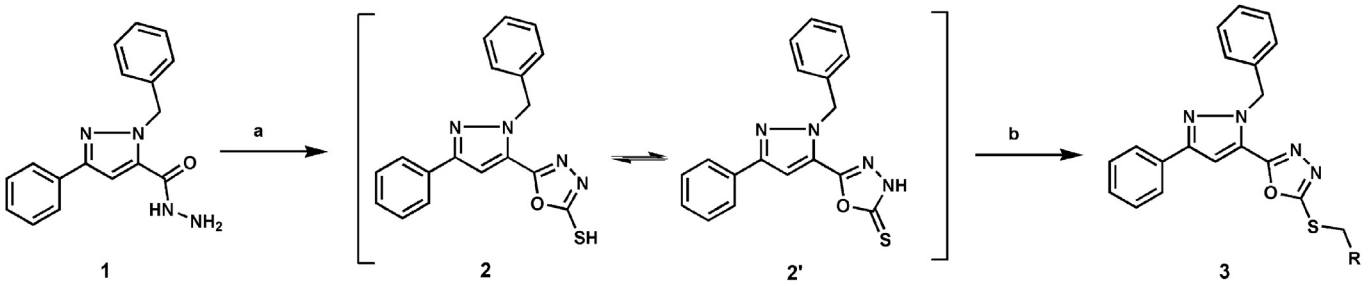

Scheme 1. Synthesis of compounds **3a**–**3l**. Reagents and conditions: (a) CS_2, KOH, MeOH, reflux; (b) RCH_2Cl, K_2CO_3, MeCN, reflux.

2. Experimental

2.1. Materials and methods

All melt points were obtained on a melting point apparatus and were uncorrected. All fluorescence spectra were recorded with a JASCO FP-6200 spectrofluorometer equipped with a 1.0 cm path length quartz cell. All pH values were measured on a Mettler Toledo S470-USP/EP Seven Excellence pH/Conductivity Metre with a combined glass electrode. ^{1}H NMR spectra were recorded with a Bruker-300 spectrometer with TMS as an internal standard in $CDCl_3/d_6$-DMSO. Infrared (IR) samples were prepared as KBr pellets, and spectra were obtained in the 400–4000 cm^{-1} range using a Thermo Scientific Nicolet iS10 FT-IR spectrometer. Elemental analyses (C, H, and N) were performed on a Perkin–Elmer Model 2400 analyzer. X-ray analysis was measured on a Bruker Avance-300 spectrometer. Reaction progress was monitored by thin layer chromatography (TLC) using ethyl acetate/peter ether as the mobile phase on pre-coated silica gel plates. All measurements were performed under ambient atmosphere at room temperature. Xanthine and bovine milk xanthine oxidase (Grade I, ammonium sulfate suspension) were purchased from Sigma–Aldrich Chemicals Co. The other chemicals were of analytical grade obtained from commercial suppliers and used without further purification.

2.2. Synthesis of the compounds

2.2.1. 5-(1-Benzyl-3-phenyl-1H-pyrazol-5-yl)-1,3,4-oxadiazole-2-thiol (*2*)

To a mixture of 1-benzyl-3-phenyl-1H-pyrazole-5-carbohydrazide **1** (2.92 g, 10 mmol) and potassium hydroxide (0.56 g, 10 mmol) in methanol (10 mL), 5 mL of carbon disulfide was added dropwise. The mixture was stirred under room temperature for 30 min and then was heated to reflux for 4 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, the solvent was removed under reduced pressure, and 100 mL of water was added. It was then neutralized with acetic acid. The solid obtained was filtered, washed with water and recrystallized from ethanol to give 2.84 g of **2** in 84.9% yield, as white solid, mp 214–215 °C; IR (KBr, pellet, cm^{-1}): *ν* 3096 (br), 3001 (w), 2941 (w), 2811 (w), 2769 (w), 1639 (m), 1507 (s), 1483 (s), 1455 (s), 1329 (w), 1305 (s), 1183 (m), 1055 (s), 963 (s), 761 (m), 730 (s), 683(m). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS, ppm): *δ* 10.52 (s, 1H, SH), 7.84–7.82 (d, 2H, ArH), 7.44–7.21 (m, 9H, ArH), 5.75 (s, 2H, NCH_2). Elemental analysis (%) calcd. for $C_{18}H_{14}N_4OS$ (334.40): C 64.65, H 4.22, N 16.75; Found: C 64.73, H 4.32, N 16.68.

2.2.2. 2-(1-Benzyl-3-phenyl-1H-pyrazol-5-yl)-5-(4-fluorobenzylthio)-1,3,4-oxadiazole (*3a*)

A mixture of **2** (1.67 g, 5 mmol), 4-fluorobenzyl chloride (1.67 g, 5 mmol) and potassium carbonate (g, 5.2 mmol) in acetonitrile (20 mL) was heated to reflux for 3 h. The resulting solution was concentrated by vacuum evaporation and the residue was poured onto crushed ice, then the solid precipitate was collected by filtration, washed with water and cold ethanol to afford pure precipitates of **3a** in 86.8% yield, as white solid, mp 149–150 °C; IR (KBr, pellet, cm^{-1}): *ν* 3124 (w), 3063 (w), 3036 (w), 2969 (w), 2948 (w), 2921 (w), 1621 (m), 1489 (s), 1471 (s), 1193 (m), 1087 (m), 1058 (m), 819 (m), 766 (s), 729 (s). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS, ppm): *δ* 7.85–7.83 (d, 2H, ArH), 7.42–7.21 (m, 12H, ArH), 7.07 (s, 1H, pyrazole), 5.90 (s, 2H, NCH_2), 4.43 (s, 2H, SCH_2). Elemental analysis (%) calcd. for $C_{25}H_{19}FN_4OS$ (442.51): C 67.86, H 4.33, N 12.66; Found: C 67.83, H 4.34, N 12.53.

2.2.3. 2-(1-Benzyl-3-phenyl-1H-pyrazol-5-yl)-5-(4-chlorobenzylthio)-1,3,4-oxadiazole (*3b*)

Prepared similarly using 4-chlorobenzyl chloride instead of 4-fluorobenzyl chloride. White solid; yield 87.4%, mp 128–129 °C; IR (KBr, pellet, cm^{-1}): *ν* 3116 (w), 3067 (w), 3036 (w), 2974 (w), 2952 (w), 2926 (w), 1619 (m), 1508 (s), 1469 (s), 1421 (m), 1222 (s), 1190 (m), 1059 (s), 828 (m), 768 (s), 729 (s), 689 (s). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS, ppm): *δ* 7.85–7.83 (d, 2H, ArH), 7.41–7.21 (m, 10H, ArH), 7.08 (s, 1H, pyrazole), 7.02–6.99 (t, 2H, ArH), 5.90 (s, 2H, NCH_2), 4.45 (s, 2H, SCH_2). Elemental analysis (%) calcd. for $C_{25}H_{19}ClN_4OS$ (458.96): C 65.42, H 4.17, N 12.21; Found: C 65.28, H 4.25, N 12.36.

2.2.4. 2-(1-Benzyl-3-phenyl-1H-pyrazol-5-yl)-5-(4-bromobenzylthio)-1,3,4-oxadiazole (*3c*)

Prepared similarly using 4-bromobenzyl chloride instead of 4-fluorobenzyl chloride. White solid; yield 84.5%, mp 154–156 °C; IR (KBr, pellet, cm^{-1}): *ν* 3124 (w), 3059 (w), 3034 (w), 2966 (w), 2948 (w), 2918 (w), 1621 (m), 1486 (s), 1471 (s), 1193 (m), 1058 (s), 1010 (m), 813 (m), 766 (s), 729 (s), 690 (s). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS, ppm): *δ* 7.85–7.84 (d, 2H, ArH), 7.47–7.40 ()7.22 (m, 12H, ArH), 7.09 (s, 1H, pyrazole), 5.91 (s, 2H, NCH_2), 4.43 (s, 2H, SCH_2). Elemental analysis (%) calcd. for $C_{25}H_{19}BrN_4OS$ (503.41): C 59.65, H 3.80, N 11.13; Found: C 59.73, H 3.74, N 11.32.

2.2.5. 2-(1-Benzyl-3-phenyl-1H-pyrazol-5-yl)-5-(4-nitrobenzylthio)-1,3,4-oxadiazole (*3d*)

Prepared similarly using 4-nitrobenzyl chloride instead of 4-fluorobenzyl chloride. White solid; yield 78.7%, mp 167–168 °C;