Synthesis of Novel 1,3,4, Oxadiazole from Chloro (amino pyrazoly) ketone carbohydrazide, their Fluorescence properties and Xanthine oxidase inhibitory activity

**Chemistry**

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**ABSTRACT**

In order to explore high strength organic electro transporting electroluminescent (EL) materials, three new pyrazole oxadiazole compounds containing the phenyl group in the 1st position of pyrazole, methyl group at 3rd position, (amino pyrazoly) ketone with 1,3,4 oxadiazole moiety contains various substituent in the 4th position and chloro group in the 5th position of pyrazole were synthesized. Their structures were characterized by IR, 1H NMR spectra and elemental analysis. A new method of synthesizing 1,3,4-oxadiazole compound was described showing fluorescence characteristic. The fluorescence properties were measured by fluorometry. And synthesized oxadiazole were tested for xanthine oxidase inhibitory activity against standard drug Allopurinol.

**KEYWORDS:**

Pyrazole-4-Carbohydrazide, Chloro (amino Pyrazoly) ketone, 1, 3, 4 Oxadiazole, Fluorescence property, XO inhibitory activity.

**Introduction**

1, 3, 4-oxadiazole has great unparalleled performance in electronic-injection and electronic transmission and possess various biological activities like antimicrobial, anti-inflammatory, antituberelar, anti-convulsant, analgesic, anti-tumor activities. In the past decades, various attempts were made to elucidate the mechanism of fluorescence in these compounds by studying the effect of substituent on the absorption and fluorescence properties of this class of compounds. The organic and polymer electroluminescence (EL) devices have show several advantages over inorganic ones, such as low cost, high luminous efficiency, wide selection of emission colors via molecular design of organic and polymer materials, and easy processing. In recent years, synthesis and study of pyrazole derivatives have become more and hotter in heterocyclic chemistry because pyrazole possesses good biological activity. Pyrazole derivatives are well known for their applications in fluorescence probes and pharmacological activities such as Antibacterial and antifungal, Antiviral, Antipretic, antioxidant, anticancer, analgesics, anti-inflammatory, antidepressant and anticonvulsant activities.

Xanthine oxidase (XO) inhibitors have been widely used for the treatment of gout. Xanthine oxidase 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. Allopurinol, a purine analogue is the first XO inhibitor and has been widely used in clinical management of gout for several decades. Xanthine oxidase inhibitors such as allopurinol interfere with the conversion of hypoxanthine to xanthine and then to uric acid. In general, allopurinol is the drug of choice; however it has been observed that allopurinol induces side effects such as fever, skin rash, eosinophilia, hepatitis and worsened renal function. Thus, new alternatives with an increased therapeutic activity and less side effects are desired. In the recent years, several synthetic skeletons containing thiazole, triazole pyrimidine have been reported to display XO inhibitory activity.

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. In this paper, three new pyrazole oxadiazole compounds were designed and synthesized. Their fluorescence properties were measured as quantum yield and the results showed that target compounds had good fluorescence and XO inhibitory activity tested of this pyrazole-based 1,3,4-oxadiazole derivatives against standard drug Allopurinol. The synthetic route of the target compounds shows in Scheme 1.

**Experimental**

**Materials and methods**

All melting points were obtained on a melting point apparatus and were uncorrected. All fluorescence spectra were recorded with a Fluorolog Modular spectrophluorometer of Horiba scientific equipped with a 2 mm path length quartz cell and using quinine sulfate as a reference substance. All absorbance spectra were recorded with a Bruker-400 spectrometer with TMS as an internal standard in CDC3/d6-DMSO. Infrared (IR) samples were prepared as KBr pellets and recorded on Perkin Elmer FTIR and only noteworthy absorption levels (cm⁻¹) are listed. Elemental analyses (C, H, N and S) were performed on a Perkin Elmer Model 2400 analyzer. Reaction progress was monitored by thin layer chromatography (TLC) using ethyl acetate/pet-ether as the mobile phase on pre-coated silica gel plates. 5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-Carboxyhydrazide was prepared as per the procedure described in our research paper.

Ethyl (ethoxymethylene) cyanoacetate, hydrazine hydrate, phosphorus oxychloride, 2-thiophene carbonyl chloride, Piperonylic acid, Cinnamic acid were purchased from Alfa Aesar, SD Fine Chem and Sigma-Aldrich. Quinine, Allopurinol, Xanthine Oxidase Enzyme (5Unit/mg) was purchased from Sigma-Aldrich.

**Synthesis of intermediates and target compounds**

5-Methyl-2-phenyl-2, 4-dihydro-pyrazole-3-one [I]  
50 gm (49 mL, 0.384 mole) of distilled ethyl acetocetate and 40 gm (36.5 mL, 0.37 mole) of phenyl hydrazine were mixed together in a 2 mm path length quartz cell. 1H NMR spectra were recorded with a Bruker-400 spectrometer with TMS as an internal standard in CDC3/d6-DMSO. Infrared (IR) samples were prepared as KBr pellets and recorded on Perkin Elmer FTIR and only noteworthy absorption levels (cm⁻¹) are listed. Elemental analyses (C, H, N and S) were performed on a Perkin Elmer Model 2400 analyzer. Reaction progress was monitored by thin layer chromatography (TLC) using ethyl acetate/pet-ether as the mobile phase on pre-coated silica gel plates. 5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-Carboxyhydrazide was prepared as per the procedure described in our research paper.
anhydrous sodium sulfate. The chloroform was evaporated to get the hydrazine hydrate layer. 25.0 mL of chloroform was added to the reaction mass and methyl ester was kept overnight under reflux in 10.0 mL of hydrazine hydrate. 2.0 gm of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid methyl ester was recrystallized in ethanol. Yield- 75 % mp: 68 °C.

5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic Acid Methylester [IV]

2.365 gm (0.01 mole) of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid was dissolved in 25 mL of ether. The solution was cooled to 0°C, with proper precaution excess diazomethane solution was added. As the reaction continued nitrogen present in the solution bubbled out. The reaction mass was kept in refrigerator overnight. Excess diazomethane was distilled out. The solid precipitate of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid methylester was recrystallized in ethanol. Yield- 75 % mp: 68 °C.

5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic Acid

2.0 gm of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid methyl ester was kept overnight under reflux in 10.0 mL of hydrazine hydrate. 25.0 mL of chloroform was added to the reaction mass and the hydrazine hydrate layer was separated. The chloroform layer was washed thrice with water. The chloroform layer was dried on anhydrous sodium sulfate. The chloroform was evaporated to get solid precipitate of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid. It was recrystallized in ethanol. Yield- (60.0 %) mp: 172-175 °C.

Ethyl-5-amino-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbonyl)-1H-pyrazole-4-carboxylate [VI]

To a solution of 12.52 gm (0.05 mol) 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid [V] in 50 mL DMF is added 12.69 gm (0.075 mol) of Ethyl (ethoxymethylene) cyanoacetate. The mixture is refluxed for 8 h and cooled to room temperature. The precipitate solid is collected by filtration, wash with ethanol and a white solid is obtained is than crystallized in ethanol to gives Ethyl-5-amino-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbonyl)-1H-pyrazole-4-carboxylate [VI]. This compound is obtained as colorless white crystals (alcohol), yield 54 %, mp 236–238°.

1H NMR (400 MHz, δ ppm, CDCl3): δ 0.85 ppm (t, 3H, COOCH3); δ 0.2 1 ppm (d, 3H, CH3); δ 3.85 ppm (q, 2H COOCH3); δ 5.45 ppm (s,2H,NH); δ 7.0–7.1 ppm (m,5H Ar),δ 7.39 ppm (s,1H, CH)

IR (potassium bromide): 900-675 - C=C stretching; 1097– CI stretching; 1252.79-1350– CH bending; 1400-1450 – CH; bending 1300-1600– Ring stretching vibration: 1590.34–C=C stretching; 1719.57–C=C stretching; 3213.53-3415.03 – Heteroaromatic N-H stretching

Anal. Calculated for C15H14N2O, C=54.63%; H=4.31%; N=18.74%
Found: C=53.51%; H=5.21%; N=18.00%

This compound is obtained as colorless solid, yield 60 %; mp 292°C.

1H NMR (400 MHz, δ ppm, DMSO): δ 2.5 ppm (s, 3H, CH3); δ 4.5 ppm (s,2H, CONH2); δ 5.5 ppm (s,2H, NH); δ 6.5–7.7 ppm (m,5H Ar); δ 8.15 ppm (s,1H, CH); δ 9.9 ppm (s,1H, NH, D O Ex.)

IR (potassium bromide): 706.92 – C=C stretching: 1622.16 – C=C stretching in ring: 1655.92, 1696.42 – C=C stretching; 3112.53, 3285.79 – NH stretching; 3432.39 – NH stretching

Anal. Calculated For C15H14N2O, C=54.41%; H=3.61%; N=15.86%
Found: C=55.10%; H=4.01%; N=16.50%

General method for the synthesis of 1, 3, 4 Oxadiazole of 5-amino-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbonyl)-1H-pyrazole-4-carboxylate [VII] A suspension of Ethyl-5-amino-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylate [VI] 7.5 gm (0.02 mol) in 25 mL 80% hydrazine hydrate is heated at 105° for 6 h. Then the solution is evaporated under vacuum and cooled to room temperature. The residue is filtrated; wash with 25 mL diethyl ether three times. A white crystalline solid of 5-amino-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbonyl)-1H-pyrazole-4-carboxylic acid. It was recrystallized in ethanol. Yield- (60.0 %) mp: 172-175 °C.

For the preparation of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic Acid [II] For the preparation of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid, POCl3 (0.35 mole, 32.0 mL.) was added to ice-cold dimethyl formamidine (0.16 mole, 12.0 mL.). To this mixture 5-methyl-2,4-dihydro-pyrazole-3-one (0.05 mole, 8.1 gm) was added and the mixture was heated under reflux for 1.0 hrs. After cooling the reaction mixture was poured into ice-cold water (300 mL.). The solid precipitated was collected by filtration, washed with cold water, dried and recrystallized from ethanol to give pale yellow crystal of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid. Yield (90 %), mp: 145–148 °C.

5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic Acid

In three 500 mL flask, 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid 0.05 mol and 100 mL of water was slowly added dropwise 0.075 mol KMnO4 in 200 mL aqueous oxidation. Dropping was completed, the temperature was raised to 70–80 °C reactor 8 h. Adjusting the pH of the reaction solution was made alkaline with 10% Barium Hydroxide solution was slowly cooled, insoluble’s were removed by filtration, and the filtrate was acidified with concentrated hydrochloric acid, the precipitated white solid was suction filtered, washing and drying, a white solid; Yield: Yield- (70.0 %) mp: 230 °C.

5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic Acid Methylester [V]

7.5 gm (0.02 mol) of 5-amino-1-(5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbonyl)-1H-pyrazole-4-carboxylate [VI] was dissolved in 15 mL of dimethyl formamide (0.16 mole, 12.0 mL.). To this mixture 5-methyl-2-phenyl-2,4-dihydro-pyrazol-3-one (0.05 mole, 8.1 gm) was added and the mixture was heated under reflux for 1.0 hrs. After cooling the reaction mixture was poured into ice-cold water (300 mL.). The solid precipitated was collected by filtration, washed with cold water, dried and recrystallised from ethanol to give pale yellow crystal of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid. Yield (90 %), mp: 145–148 °C.
Fluorescent spectra characteristics of compound VIII A is not ideal when the solvent is DMF. But it is ideal in case of compound VIII B and VIII C, which showing good fluorescent spectra in DMF.

The maximum fluorescence intensity and fluorescence yield in chloroform is the best it may be caused by the polar of compound A matching with the polarity of chloroform, however, the polarity of DMF is high, the dissolvability of compound VIII A in these solvents is not very good, so the fluorescent spectra characteristics of compound VIII A is not ideal when the solvent is DMF. But it is ideal in case of compound VIII B and VIII C, which showing good fluorescent spectra in DMF.
mM xanthine solution (prepare 100 mL by initially dissolving Xanthine by adding 2-3 drops of 1.0 M NaOH to increase the solubility. Add approximately 90 mL of deionized water. Adjust to pH 7.5 at 25°C with either 1 M NaOH or 1 M HCl. Dilute to final volume of 100 mL. 0.05 units/mL of XO Enzyme solution (Immediately before use, prepare a solution containing 0.05 units/mL of Xanthine Oxidase in cold 50 mM Potassium Phosphate buffer).

The total volume of the assay mixture was 3.2 mL and consisting of 1 mL sample solution studied, 1 mL 0.15 M phosphate buffer (pH 7.4), 100 µL of the enzyme xanthine oxidase solution. After preincubation of the test solution at 37°C for 15 min, the reaction was initiated by addition 100 µL of xanthine substrate solution and incubated at 37°C for 30 min. The reaction was stopped by adding 1 mL of 1N HCl. The absorption was measured at 295 nm to indicate the formation of uric acid. All experiments were performed in triplicate and averaged. The percentage inhibitory activity of the samples were determined against a DMSO blank, and calculated by using the following formula. Allopurinol was used as Standard. Inhibition (%) = 100 - [(OD test compound /OD control) × 100]

Table 2: In Vitro Xanthine oxidase inhibitory activity of compounds VIII (A-C) and Allopurinol

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>XO Inhibitory activity IC50 (µM)</th>
<th>a</th>
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<tbody>
<tr>
<td>VIII A</td>
<td></td>
<td>75.60</td>
<td></td>
</tr>
<tr>
<td>VIII B</td>
<td></td>
<td>50.55</td>
<td></td>
</tr>
<tr>
<td>VIII C</td>
<td></td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>Allopurin</td>
<td></td>
<td>2.6</td>
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</table>

Conclusions

Dissolvability of compound VIII A is showing good fluorescence spectra in Chloroform, so shows good fluorescence emission and the fluorescence spectra characteristic is best with this concentration. Fluorescence Quantum Yield is 0.27. Same as the compound VIII B & VIII C shows good fluorescence emission using DMF as a Solvent. The results showed that the target compounds had good fluorescence and λ em ranged from 410 nm to 550 nm and fluorescence quantum yields up to 0.48.

In addition, the in vitro inhibitory activity against xanthine oxidase was evaluated and compound VII A and VIII B was found to be the most active against commercial XO with IC50 value 75.60 mM and 50.55 mM respectively. Although the inhibitory activity was lower compared to that of the standard drug allopurinol, further optimization of substituted R groups which may obtain more potent XO inhibitors are in progress in our laboratory.

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References