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# Synthesis of novel nitric oxide donating pyrazoles

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## Abstract

A series of 1-aryl-5-(4-hydroxy) phenyl pyrazoles was synthesized via reaction of various aromatic chalcones with substituted phenylhydrazine in ethanol. Nitric oxide donor group i.e., nitrate was introduced by treating synthesized pyrazoles with a mixture of fuming nitric acid and acetic anhydride in chloroform in cold conditions. Nitric oxide has low solubility in water and is unstable in the presence of various oxidants. It makes it difficult to introduce as such into biological systems in a controlled or specific fashion. Therefore, development of chemical agents that release NO is important. Pyrazole compounds have been identified as potent COX inhibitors with good pharmacokinetic profiles. These reports prompted us to undertake the synthesis of some novel pyrazoles with NO-donating properties.

**Keywords**: nitric oxide, pyrazoles, phenyl, phenylhydrazines.

## Introduction

Compounds containing pyrazole moiety as a core structure exhibit a wide range of biological Activities (Stauffer et al. 2000, Sawyer et al. 2003, Chimenti et al. 2003, Singh et al. 2004, Pevarello et al. 2005). Nitric Oxide has low solubility in water and is unstable in the presence of various oxidants. It makes it difficult to introduce as such into biological systems in a controlled or specific fashion. Therefore development of chemical agents that release NO is important. Pyrazole compounds have been identified as potent COX inhibitors with good pharmacokinetic profiles. These reports prompted us to undertake the synthesis of some novel pyrazoles with NO-donating properties. Combining of two bioactive molecules (Pyrazole and NO) into one structure with enhanced biological activities.

## Materials and Methods

Chalcones (Fig. 1) were synthesized by a base catalysed Claisen-Schmidt condensation reaction of appropriately substituted aromatic acetophenones (1-2) and substituted aromatic aldehydes (3-5) in the presence of 10% NaOH in ethanol (Ezawa et al. 2005). Heating at reflux chalcones with phenylhydrazine in absolute ethanol yielded corresponding pyrazoles. Treatment of pyrazoles with 4-Chlorobutanol in DMF afforded the corresponding substituted pyrazoles. Nitric oxide donor group i.e., nitrate was

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introduced by treating with a mixture of fuming nitric acid and acetic anhydride in chloroform in cold condition.

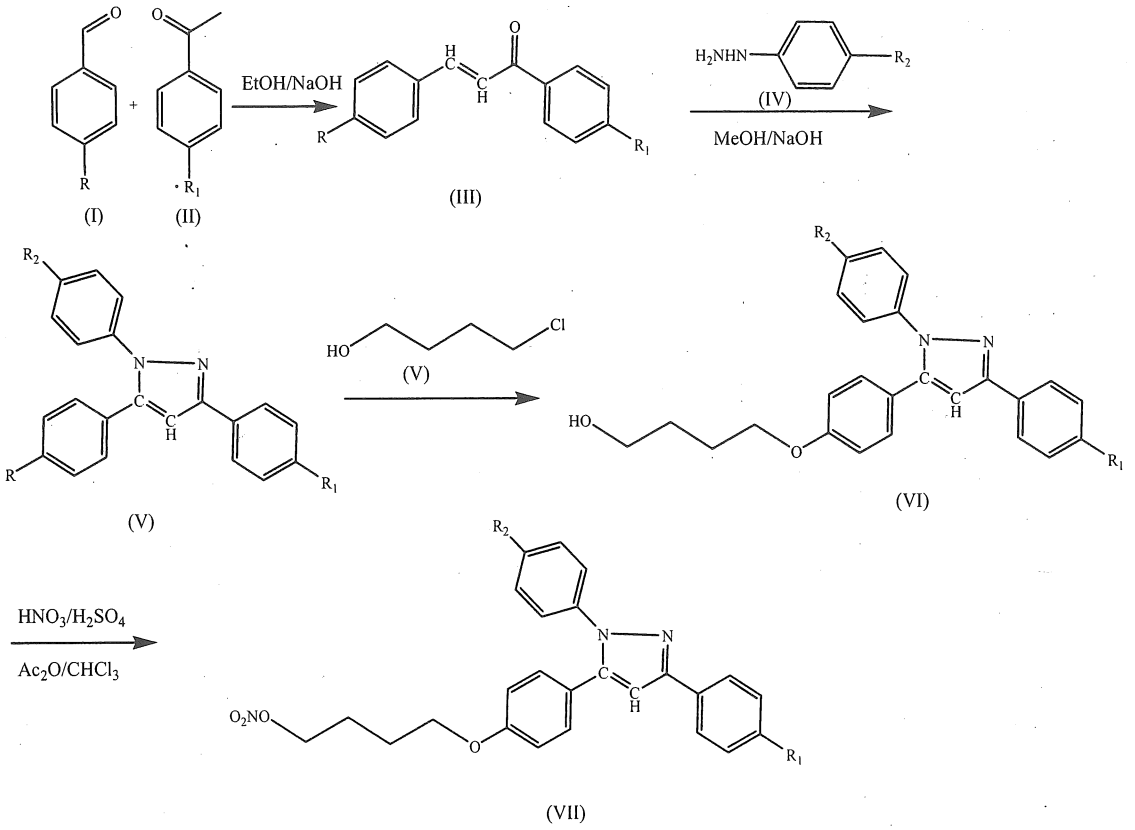


**Figure 1.** Synthesis of different NO-donating pyrazole derivatives

Reagents: i) 10% NaOH, EtOH; ii) $C_6H_5NHNH_2$, MeOH; iii) DMF,; iv) $HNO_3$, $H_2SO_4$, $Ac_2O$

The structure of prepared compounds was confirmed on the basis of their IR, 1H NMR and UV spectroscopical analysis.

*Nitric oxide release measurement*

The NO-releasing properties of the tested compounds were assessed in both phosphate buffer of pH 7.4 and pH 1 using 0.1 N HCl with Griess reagent (Khaled et al. 2007, Manojkumar et al. 2009). The reaction was carried out in the presence of N-acetylcysteine as a source of the SH group. The amount of NO released from the tested compounds was measured relative to NO released from standard sodium nitrite solution, calculated as the amount of NO (mol/mol) released and are listed in Table 1.

**Table 1.** The amount of NO released from tested compounds 7a, 7b, 7c, 7d and 7e at pH 7.4

| Compound No. | Amount of NO released (mol/mol)±SEM | | | |
|---|---|---|---|---|
| | 1 h | 2 h | 3 h | 4 h |
| 7a | 0.109 ± 0.050 | 0.202 ± 0.050 | 0.287± 0.050 | 0.325± 0.050 |
| 7b | 0.113 ± 0.050 | 0.204± 0.050 | 0.302± 0.050 | 0.304± 0.050 |
| 7c | 0.024 ± 0.050 | 0.046± 0.050 | 0.121± 0.050 | 0.223± 0.050 |
| 7d | 0.122 ± 0.050 | 0.234± 0.050 | 0.301± 0.050 | 0.404± 0.050 |
| 7e | 0.117 ± 0.050 | 0.243± 0.050 | 0.333± 0.050 | 0.264± 0.050 |

## Results and Discussion

The IR spectra of the prepared pyrazoles showed ν (C=N) stretching at 1611-1599 $cm^{-1}$ due to ring closure, absorption bands at 1115-1280 $cm^{-1}$ due to (C-N) stretching vibrations confirming the formation of pyrazole ring. The sharp band in region of 3226-3335 $cm^{-1}$ due to (-NH-) was also seen. The $^1H$ NMR in $CDCl_3$ was also observed e.g. doublets of doublets at δ 3.20-3.44, 3.40-3.53 and 5.04-5.27 ppm ($J_{HA-Hb}$= 16.50-16.70, $J_{HA-HX}$= 7.30-7.90, $J_{Hb-HX}$=9.90-10.20 Hz), for the protons Ha, Hb and Hx as protons of pyrazole ring. Pyrazoles from substituted acetophenones carrying highly electronegative groups (4-Chloro) made Ha and Hb that appeared to be equivalent. The $-CH_2$ protons appeared as doublet at δ 3.24 and 3.44 ppm while the CH proton appeared as triplet at δ 5.80 ppm.

Heating at reflux of chalcones with phenylhydrazine in absolute ethanol gave the corresponding pyrazoles. The disappearance of ketonic stretching band and appearance of (C=N) stretching band at 1608 $cm^{-1}$ in the IR spectra confirmed the proposed structure.

The results of NO-releasing properties of the tested compounds 7a, 7b, 7c, 7d and 7e indicated that starting chalcones and pyrazole derivatives didn't release any amount of NO neither at phosphate buffer of pH 7.4 nor at pH 1 and this is a proof that the nitrates are the main sources of NO release.

NO-donating pyrazole derivatives 7a-7e was found to release different amounts of NO at phosphate buffer of pH 7.4 (Table 1). For the first 7a, 7c and 7d there was a regular increase in the amount of NO released reaching their maximum after 4 h. The group 7b and 7e were found to release a moderate amount of NO reaching their maximum after 3 h and then decreased in 4$^{th}$ h. On the other hand there is no release of NO at pH 1 and this may support the fact that NO-donating moieties are weakly hydrolyzed in the gastric lumen.

*Nitric oxide release assay*

A solution of the compound (25μg/mL) in dimethylsulfoxide (DMSO) was added to 2 mL of 1:1 v/v mixture of 50mM phosphate buffer (pH 7.4) with methanol, containing L-cysteine. The final concentration of drug was made $10^{-4}$ M. After 1 h at 37°C, 1 mL of the reaction mixture was treated with 250 μL of Griess reagent in distilled water (up to 100 mL). After 10 minutes at room temperature, the absorbance was measured at λ=548nm. The calibration curve was constructed with sodium nitrite standard solution (10-80 mol/mL). The same procedure was repeated using different solutions of the test compounds under the same conditions using 0.1 N HCl of pH 1 instead of pH 7.4.

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