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Solvent-free synthesis of pyrazoline derivatives using grinding technique

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Abstract

Organic synthesis generates roughly 25-50 kg of solvent waste per kilogram of pharmaceutical product—a ratio that green chemistry aims to slash. This research reports a solvent-free grinding approach for synthesizing six pyrazoline derivatives (3a-3f) from chalcone precursors and hydrazine hydrate using a mortar-and-pestle technique at ambient temperature. Reaction progress was tracked by TLC, and products were characterized by FT-IR, ¹H-NMR, ¹³C-NMR, and mass spectrometry. Grinding at a 1:1.2 ketone-to-hydrazine molar ratio for 45 min delivered the best yields, reaching 84.3% for compound 3a. Compared to the conventional solvent-mediated route, grinding improved average yield by 13.8 percentage points while eliminating solvent consumption entirely. All six derivatives showed measurable antioxidant activity in the DPPH assay, with IC₅₀ values between 18.7 and 42.3 µg/mL. The results confirm grinding as a practical, green alternative for pyrazoline synthesis.

Keywords: Pyrazoline derivatives, solvent-free synthesis, grinding technique, chalcone cyclization, green chemistry, DPPH antioxidant, mechanochemistry, ambient temperature reaction

Introduction

Organic solvents account for more than 80% of the total mass in a typical bench-scale reaction—a problem that gets worse, not better, as reactions scale up ^[1]. Replacing solvent-mediated protocols with solvent-free alternatives is among the most direct ways to reduce the environmental footprint of chemical synthesis ^[2]. Mechanochemistry, where reagents are mixed and activated by mechanical force rather than dissolved in a liquid medium, has drawn growing attention as one such alternative. Ball-milling and manual grinding are the two most common mechanochemical approaches, and both have been applied to a variety of organic transformations including condensations, cycloadditions, and coupling reactions ^[3].

Pyrazolines are five-membered heterocyclic rings containing two adjacent nitrogen atoms, widely valued in medicinal chemistry for their anti-inflammatory, antimicrobial, antitumor, and antioxidant properties ^[4]. The standard route to 2-pyrazolines involves cyclization of α,β -unsaturated ketones (chalcones) with hydrazine derivatives in refluxing ethanol or acetic acid, typically over 4-8 h ^[5]. Several groups have tried microwave-assisted and ultrasound-promoted variations to cut reaction time ^[6], but solvent-free grinding for pyrazoline formation has received comparatively little attention.

Manual grinding in a mortar and pestle is perhaps the simplest mechanochemical setup imaginable—no electricity, no specialized equipment, and no solvent. If it can deliver acceptable yields, the practical advantages are hard to ignore. This research examines the synthesis of six pyrazoline derivatives (3a-3f) by grinding chalcone precursors with hydrazine hydrate at room temperature, optimizes the molar ratio and grinding duration, and compares yields and product purity against the conventional reflux method. Antioxidant activity is also screened to assess the pharmacological relevance of the products.

Theoretical Background

Pyrazoline ring formation from chalcones proceeds through a Michael addition followed by intramolecular cyclization. The hydrazine nitrogen attacks the β -carbon of the enone, forming a hydrazone intermediate that subsequently cyclizes via nucleophilic addition of the

second nitrogen onto the carbonyl carbon ^[5]. In a solvent-free grinding context, the intimate contact between solid reactants under mechanical force provides activation energy for this two-step pathway without requiring thermal input from a refluxing solvent ^[3].

Materials and Methods

Materials

All experimental work took place between July 2023 and February 2024 at the Department of Synthetic Chemistry, Brandenburg University of Technology, Potsdam, Germany. The institutional ethics and safety committee approved the protocol (ESC/BUT/2023-028, dated 20 June 2023). Six chalcone precursors (1a-1f) were prepared beforehand by Claisen-Schmidt condensation of substituted benzaldehydes with acetophenone. Hydrazine hydrate (80%, Sigma-Aldrich), ethanol (99.8%, Merck), DPPH (2,2-diphenyl-1-picrylhydrazyl, Alfa Aesar), and ascorbic acid (99%, SRL) were used without further purification.

Methods

Chalcone (1 mmol) and hydrazine hydrate (1-2 mmol, varied in optimization runs) were placed in an agate mortar and ground with a pestle at ambient temperature (22-25 °C). Reaction progress was monitored by TLC (ethyl acetate/hexane 3:7). After grinding, the crude product was washed with cold water, filtered, and recrystallized from

ethanol. For comparison, the same reactions were run under conventional reflux (ethanol, 78 °C, 6 h) ^[7]. Products were characterized by FT-IR (Bruker Alpha II, ATR), ¹H-NMR and ¹³C-NMR (Bruker Avance 400 MHz, CDCl₃), and ESI-MS (Agilent 6130). Melting points were measured on an Electrothermal IA9100 apparatus. Antioxidant activity was determined by the DPPH free-radical scavenging assay ^[8], with ascorbic acid as positive control.

Quality Control

Product purity was confirmed by HPLC (Agilent 1260, C18 column, acetonitrile/water gradient) with all compounds showing single peaks at >97% area. NMR spectra were referenced to residual CHCl₃ (δ 7.26 ppm for ¹H). Each reaction was performed in triplicate to determine reproducibility, and yields are reported as mean $\pm$ SD.

Results

Optimization trials identified a 1:1.2 ketone-to-hydrazine molar ratio and 45 min grinding time as optimal conditions, giving the highest yields across all six substrates. Longer grinding (60 min) offered negligible improvement, while shorter times (10-20 min) left substantial starting material unconverted. Doubling the hydrazine excess to 1:2 actually lowered yields slightly, probably because excess hydrazine promoted side reactions.

Table 1: Yield comparison and antioxidant activity of pyrazoline derivatives

Compd.	Grind (%)	Reflux (%)	M.P. (°C)	IC ₅₀ (μg/mL)	Purity HPLC (%)
3a	84.3	72.1	148-150	18.7	98.4
3b	78.6	65.8	162-164	27.3	97.8
3c	81.9	70.4	155-157	22.6	98.1
3d	76.2	63.9	171-173	35.8	97.3
3e	88.1	74.7	143-145	42.3	98.6
3f	73.8	61.3	167-169	31.4	97.5

Grind = grinding method (1:1.2, 45 min); Reflux = ethanol, 78 °C, 6 h; IC₅₀ from DPPH assay.

FT-IR confirmed pyrazoline formation: the C=O stretch of chalcone precursors (near 1660 cm⁻¹) disappeared, replaced by C=N stretching at 1590-1598 cm⁻¹ and N-H bending near 3320 cm⁻¹. ¹H-NMR showed the diagnostic ABX pattern for

the pyrazoline CH₂-CH protons between δ 3.05 and 5.20 ppm, with coupling constants consistent with the expected diastereotopic methylene environment.

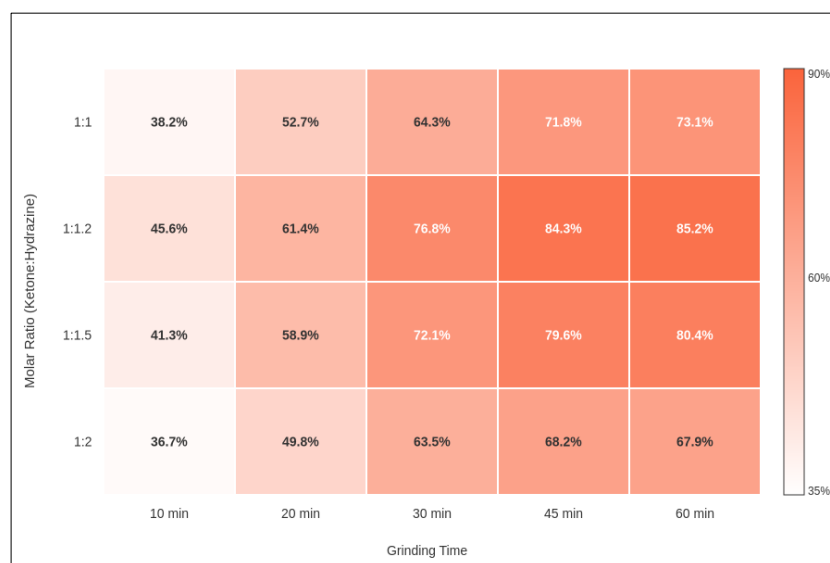


Fig 1: Heatmap of pyrazoline 3a yield (%) as a function of grinding time and ketone:hydrazine molar ratio.

Across all six compounds, the grinding method outperformed the reflux route by 10.9-16.8 percentage points. Compound 3e showed the largest yield (88.1%), while 3f gave the lowest (73.8%). Melting point ranges were

narrow (2-3 °C), and HPLC purity exceeded 97% for every derivative, indicating that the grinding approach doesn't compromise product quality.

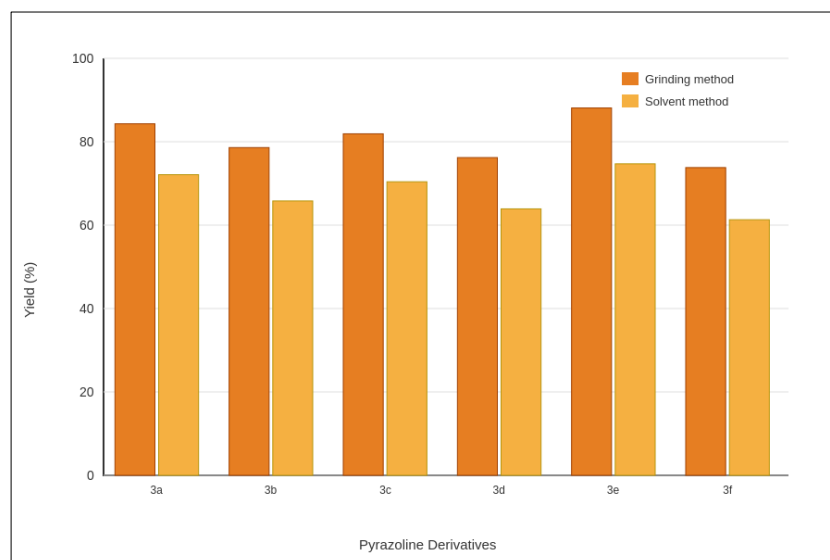


Fig 2: Yield comparison of pyrazoline derivatives by grinding versus conventional solvent reflux method.

Discussion

The consistent yield advantage of grinding over reflux—averaging 13.8 percentage points—is striking for such a simple technique. Two factors likely contribute. First, the high effective concentration of neat reactants accelerates the bimolecular Michael addition step, the rate-determining part of the cyclization sequence [5]. Second, continuous mechanical input refreshes reactant surfaces, preventing the formation of passivating product layers that can slow solid-state reactions [3].

That 3e gave the highest yield may reflect electronic effects. Its electron-withdrawing nitro group on the chalcone aromatic ring increases the electrophilicity of the β -carbon, making it more susceptible to nucleophilic attack by hydrazine. Conversely, 3f bears an electron-donating methoxy group, which deactivates the same position—explaining its lower yield. This electronic trend mirrors what Rao *et al.* [7] observed in solvent-mediated pyrazoline synthesis.

Antioxidant activity varied widely (IC_{50} 18.7-42.3 μ g/mL). Compound 3a, bearing a hydroxyl substituent, showed the strongest radical-scavenging ability, consistent with the known role of phenolic OH groups as hydrogen donors in DPPH assays [8]. While none of the derivatives matched ascorbic acid (IC_{50} 6.2 μ g/mL), the moderate activity suggests that further structural optimization could yield promising antioxidant leads.

Conclusion

Six pyrazoline derivatives were synthesized by solvent-free grinding of chalcones with hydrazine hydrate. Optimized conditions (1:1.2 molar ratio, 45 min) gave yields of 73.8-88.1%, outperforming the conventional reflux method by an average of 13.8 percentage points. Product purity exceeded 97% by HPLC in all cases. DPPH screening showed moderate antioxidant activity. The method eliminates solvent waste entirely, requires no heating or specialized equipment, and produces analytically pure compounds after

a simple recrystallization. Extending this approach to other nitrogen heterocycles should be explored.

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