Eco-Friendly Synthesis, Characterization, and Biological Evaluation of Novel Pyrazole Derivatives

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Abstract

An eco-friendly, solvent-free methodology for synthesizing pyrazole derivatives has been developed using a organo-catalyst. The final condensation of preformed intermediates with aromatic aldehydes under grinding conditions furnished the desired products in high yields within minutes. All compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The synthesized derivatives were evaluated for their antibacterial efficacy against *Escherichia coli* and antifungal activity against *Aspergillus niger*, demonstrating promising biological properties. Compared to conventional acid/base-catalyzed methods, the solvent-free approach offered significant improvements in yield, reaction time, and environmental sustainability. This expanded study further includes comparative biological profiles and synthetic efficiencies for a range of substituents and evaluates the green protocol versus conventional methods.

Keywords- Grinding method, solvent free, pyrazole, biological applications, green approach.

1. Introduction

Pyrazole and its derivatives are among the most extensively studied five-membered nitrogen-containing heterocycles in medicinal chemistry. Characterized by a 1,2-diazole ring, pyrazoles exhibit a remarkable range of biological activities including antimicrobial, anti-inflammatory, anticancer, antitubercular, antioxidant, and analgesic effects [1–3]. Several clinically approved drugs, such as celecoxib (anti-inflammatory), rimonabant (anti-obesity), and fipronil (insecticide), feature the pyrazole core, further emphasizing its pharmacological importance [4].

The wide scope for substitution at multiple positions on the pyrazole ring enables fine-tuning of physicochemical and biological properties. Electron-withdrawing and electron-donating substituents can modulate target affinity, metabolic stability, and lipophilicity, making the pyrazole nucleus a privileged scaffold in drug design [5,6]. However, traditional synthesis of pyrazole derivatives often involves multistep protocols, elevated temperatures, organic solvents, and hazardous base or acid catalysts. These factors pose environmental, safety, and economic challenges, especially when considered at scale [7].

In recent years, green chemistry has emerged as a critical paradigm in synthetic organic chemistry, emphasizing sustainability, energy efficiency, and the reduction of hazardous waste [8,9]. Among various eco-friendly strategies, solvent-free synthesis and mechanochemistry (grinding-assisted reactions) have garnered significant interest. These techniques eliminate or minimize solvent use, reduce energy consumption, and offer high atom economy — aligning well with the 12 principles of green chemistry [10,11]. Additionally, organo-catalysts such as

L-proline have proven effective in promoting condensation and cyclization reactions under mild conditions [12].

The biological relevance of pyrazole derivatives further reinforces the need for cleaner synthetic routes. Studies have shown that halogenated and nitro-substituted pyrazoles display enhanced antimicrobial and antifungal properties, potentially due to better cell permeability and enzyme inhibition [13–15]. Therefore, combining green synthetic methods with biologically relevant structural design provides a dual advantage: sustainability and therapeutic promise.

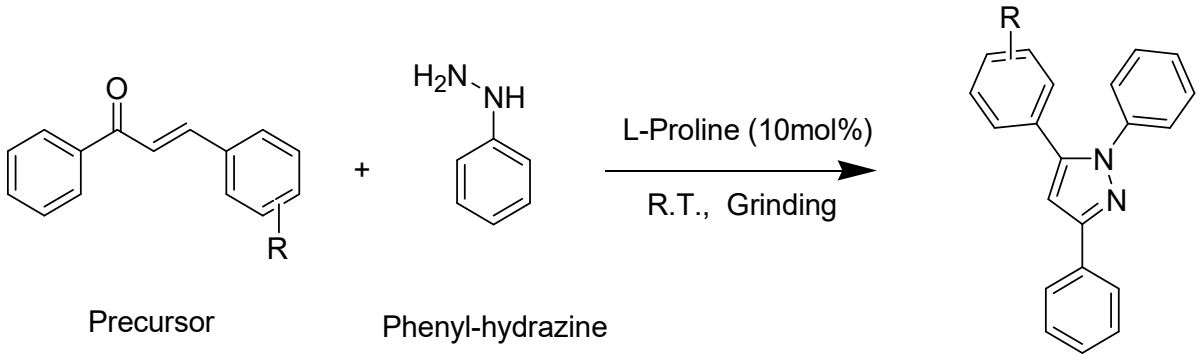
2. Synthesis of Pyrazole Derivatives

General Procedure:

The target pyrazole derivatives were synthesized via a solvent-free, catalyst-assisted method using equimolar amounts of preformed hydrazine intermediates and the precursor molecule. The precursor was synthesized by reacting acetophenone with substituted benzaldehyde. A catalytic amount of L-proline (10 mol%) was added to the reaction mixture, and the components were thoroughly ground using a mortar and pestle at room temperature for 10–15 minutes. Reaction progress was monitored by thin-layer chromatography (TLC).

Upon completion, ethanol was used to dissolve the crude product for ease of filtration and recrystallization. The desired compounds were obtained in high purity and yield without the use of solvents during the reaction step. This protocol eliminates volatile organic solvents, external heating, and high energy input, aligning well with the principles of green chemistry. The solid L-proline catalyst activates the aldehyde component, facilitating nucleophilic attack and cyclization under ambient conditions. This practical, scalable, and environmentally benign approach provides an effective route to bioactive pyrazole derivatives. [16].

The heterogeneous organic catalyst activates the aldehyde by protonation of the carbonyl oxygen, facilitating nucleophilic attack and cyclization. Combined with mechanochemical energy from grinding, this method offers a practical, scalable, and eco-friendly protocol for accessing biologically relevant pyrazole frameworks [17].



R= 4-Cl, 3-NO_2, 4-NO_2, 4-OH, 4-OCH_3, H

Scheme:I – Solvent-free synthesis of pyrazole derivatives using L-proline as a catalyst under ambient grinding conditions.

Entry	R	Yield(%)	Time(min.)	M.P.(Celsius)
4a	4-Cl	92	10	236–238
4b	3- NO_2	90	12	243–245
4c	4-NO_2	91	11	238–240
4d	4- OH	89	10	229–231
4e	4-OCH_3	88	10	232–234
4f	H	86	11	295-298

Table -1 : Synthesized Derivatives

4. Comparison between Conventional and Greener methods

To evaluate the efficiency and sustainability of the developed green method, a systematic comparison was conducted with conventional acid/base-catalyzed synthesis and grinding (green) protocols for the same set of pyrazole derivatives.

In the conventional method, NaOH was used as the catalyst in ethanol under reflux for 2–3 hours. This approach required extended heating, generated higher solvent waste, and provided only moderate yields (65–75%). Moreover, work-up and purification were more labor-intensive, leading to increased processing time and environmental burden. Another conventional method employed acetic acid in aqueous ethanol and reduced the reaction time to about 130-150 minutes. Yields were improved (78–87%) compared to above used method.

In contrast, the present solvent-free protocol, using L-proline as a heterogeneous solid organo-catalyst under grinding conditions, offered several distinct advantages:

- Drastically reduced reaction time (10–12 min)
- Higher yields (88–92%)
- No solvent usage during the reaction
- Minimal energy input, as the process proceeds at room temperature
- Easy product isolation and excellent atom economy

These results highlight the superior environmental and synthetic performance of the proposed method, making it a reliable and scalable option for green heterocycle synthesis [18-20].

Entry	Substituent	Method	Catalyst	Solvent	Time	Yield (%)	Eco Impact
4a	4-Cl	Conventional	NaOH	Ethanol	2–3 hrs	74	High waste, energy
			Acetic Acid	Aq. ethanol	130 min	85	Moderate
		Green (This Work)	L-proline	None	10 min	92	Very Low
4b	3-NO_2	Conventional	NaOH	Ethanol	2–3 hrs	70	High
			Acetic Acid	Aq. ethanol	130 min	84	Moderate
		Green (This Work)	L-proline	None	12 min	90	Very Low
4c	4-NO_2	Conventional	NaOH	Ethanol	2–3 hrs	75	High
			Acetic Acid	Aq. ethanol	150 min	87	Moderate
		Green (This Work)	L-proline	None	11 min	91	Very Low
4d	4-OH	Conventional	NaOH	Ethanol	2–3 hrs	65	High
			Acetic Acid	Aq. ethanol	140 min	78	Moderate
		Green (This Work)	L-proline	None	10 min	89	Very Low
4e	4-OCH_3	Conventional	NaOH	Ethanol	2–3 hrs	68	High
			Acetic Acid	Aq. ethanol	130 min	80	Moderate
		Green (This Work)	L-proline	None	10 min	88	Very Low
4f	-H	Conventional	NaOH	Ethanol	2–3 hrs	66	High
			Acetic Acid	Aq. ethanol	140 min	79	Moderate
		Green (This Work)	L-proline	None	10 min	90	Very Low

Table -2: Comparative analysis of conventional and solvent-free methods for the synthesis of pyrazole derivatives.

5. Characterization of Representative Compound

All synthesized pyrazole derivatives were structurally confirmed using FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry.In the IR spectra, characteristic absorptions were observed in the range of 3125–3135 cm^{-1} for Aromatic C–H stretching, 1575–1595 cm^{-1} for C=N, and 1030-1040 for N-N confirming the core pyrazolone framework.

The ^{1}H NMR spectra of all compounds showed aromatic protons as multiplets in the δ 7.0–8.0 ppm range, with methoxy substituents reflected as singlets at δ 2.0–3.8 ppm depending on the group.

The ^{13}C NMR spectra confirmed the presence of imine (C=N), and aromatic carbon signals, with chemical shifts consistent across the series. Methyl and methoxy carbons were also clearly distinguished in aliphatic regions.

Mass spectra (ESI-MS) for all compounds showed molecular ion peaks [M^+] in accordance with the expected molecular weights.

Compound 4a:

IR (KBr, cm^{-1}):ν(ArC-H) 3130, ν(C=N) 1580, ν(N–N) 1030, ν(C–Cl) 780.^{1}H NMR (500 MHz, DMSO-d_6): δ 7.23–7.67 (11H, m, aromatic H), 7.93 (2H, m, aromatic H), 8.09 (2H, m, aromatic H).^{13}C NMR: δ 108.1, 123.3, 125.1, 127.3, 127.6, 128.4, 128.6, 129.2, 133.8, 134.6, 137.7, 145.3, 149.9.MS (ESI): m/z = 331 [M^+].

Compound 4b:

IR (KBr, cm^{-1}):ν(ArC–H) 3132, ν(C=N) 1576, ν(N–N) 1040, ν(NO_2) 1520–1340.^{1}H NMR (500 MHz, DMSO-d_6): δ 7.40–7.83 (9H, m, aromatic H), 7.91–8.20 (5H, m, aromatic H), 8.38 (1H, d, aromatic H).^{13}C NMR : δ 108.1, 119.3, 123.3, 123.9, 125.1, 127.6, 128.4, 128.6, 129.2, 131.2, 133.8, 134.6, 137.7, 145.3, 148.9, 149.9.MS (ESI): m/z = 342 [M^+].

Compound 4c:

IR (KBr, cm^{-1}):ν(ArC–H) 3135, ν(C=N) 1578, ν(N–N) 1035, ν(NO_2) 1520–1345.^{1}H NMR (500 MHz, DMSO-d_6): δ 7.41–7.75 (7H, m, aromatic H), 7.90–8.14 (6H, m, aromatic H), 8.22 (2H, d, aromatic H).^{13}C NMR: δ 108.1, 123.3, 124.8, 125.1, 127.1, 127.6, 128.4, 128.6, 129.2, 133.8, 137.7, 145.3, 147.9, 149.9.MS (ESI): m/z = 342 [M^+].

Compound 4d:

IR (KBr, cm^{-1}):ν(ArC–H) 3125, ν(C=N) 1578, ν(N–N) 1038, ν(O–H) 3400 (broad).^{1}H NMR (500 MHz, DMSO-d_6): δ 7.16 (2H, d, J = 8.8 Hz, Ar–H), 7.27 (1H, s, Ar–H), 7.38–7.66 (6H, m, Ar–H), 7.73 (2H, d, J = 8.9 Hz, Ar–H), 7.92 (2H, d, J = 8.2 Hz, Ar–H), 8.06 (2H, d, J = 7.8 Hz, Ar–H).^{13}C NMR: δ 108.1, 115.3, 123.3, 125.1, 127.6, 128.0, 128.4, 128.6, 129.2, 133.8, 137.7, 145.3, 149.9, 157.1.MS (ESI): m/z = 313 [M^+].

Compound 4e:

IR (KBr, cm^{-1}):ν(ArC–H) 3133, ν(C=N) 1575, ν(N–N) 1038, ν(C–O) 1240.^{1}H NMR (500 MHz, DMSO-d_6): δ 3.80 (3H, s, OCH_3), 7.07 (2H, d, J = 8.9 Hz, Ar–H), 7.25 (1H, s, Ar–H), 7.37–7.64 (6H, m, Ar–H), 7.72 (2H, d, J = 8.9 Hz, Ar–H), 7.92 (2H, d, J = 8.2 Hz, Ar–H), 8.06 (2H, d, J = 7.7 Hz, Ar–H).^{13}C NMR: δ 55.3 (OCH_3), 108.1, 114.0, 123.3, 125.1, 127.6, 128.4, 128.6, 129.0, 129.2, 133.8, 137.7, 145.3, 149.9, 160.3.MS (ESI): m/z = 327 [M^+].

Compound 4f :

IR (KBr, cm^{-1}):ν(ArC–H) 3129, ν(C=N) 1607, ν(N–N) 1025.^{1}H NMR (500 MHz, DMSO-d_6): δ 7.37–7.68 (10H, m, aromatic H), 7.75 (2H, d, Ar–H), 7.94 (2H, d, Ar–H), 8.10 (2H, d, Ar–H).^{13}C NMR: δ 108.1, 123.3, 125.1, 126.4, 127.6, 128.4, 128.6, 128.8, 129.2, 133.8, 137.7, 145.3, 149.9.MS (ESI): m/z = 297 [M^{+}].

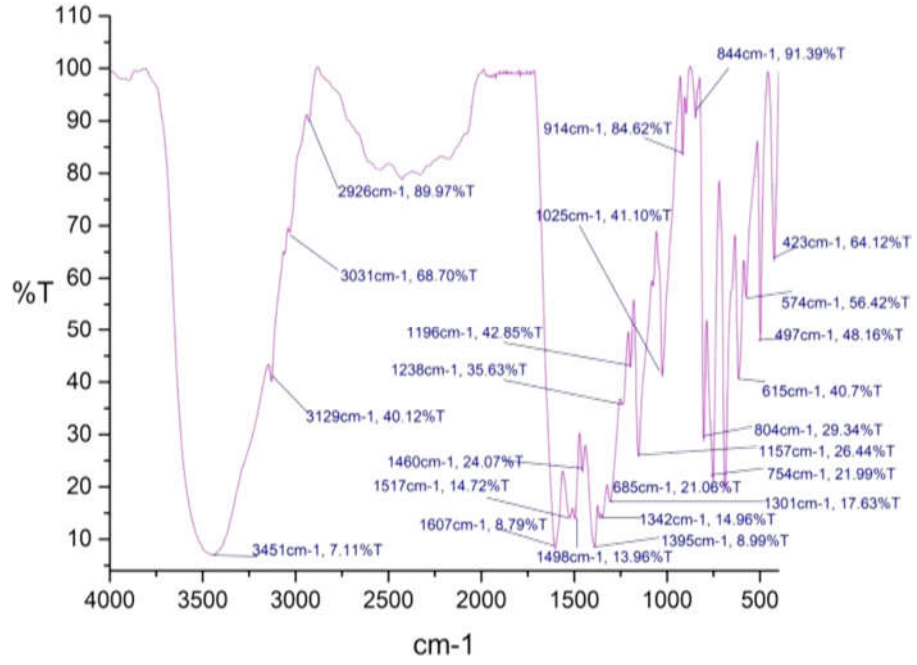


Figure 1: IR spectra of 1,3,5-triphenyl-1H-pyrazole (4f).

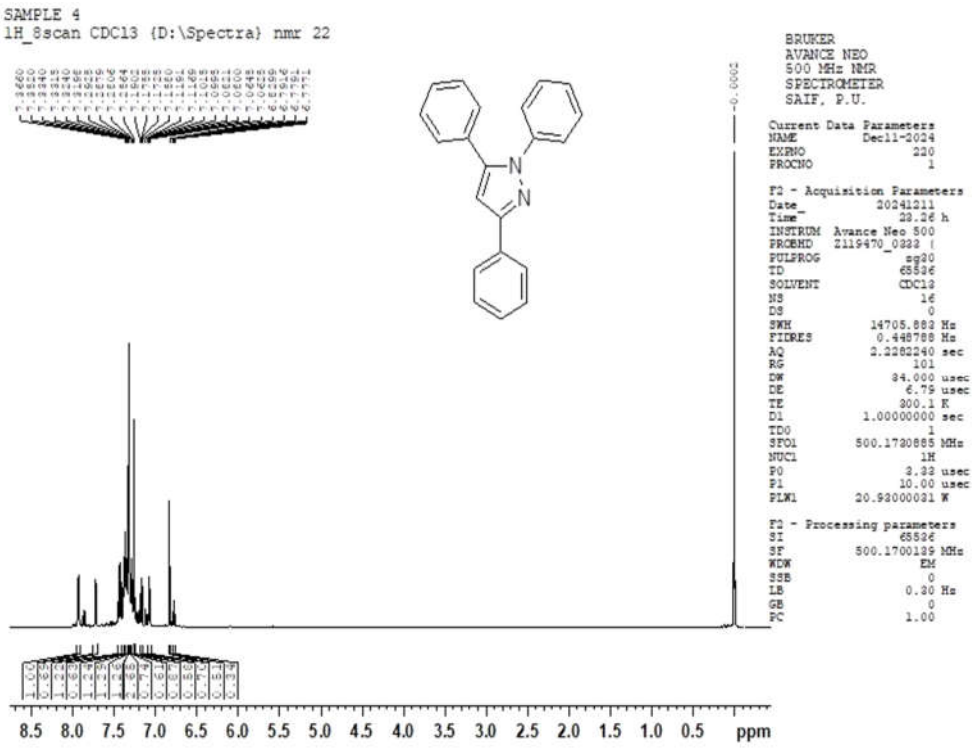


Figure 2:^{1}H NMR spectra of 1,3,5-triphenyl-1*H*-pyrazole (4f).

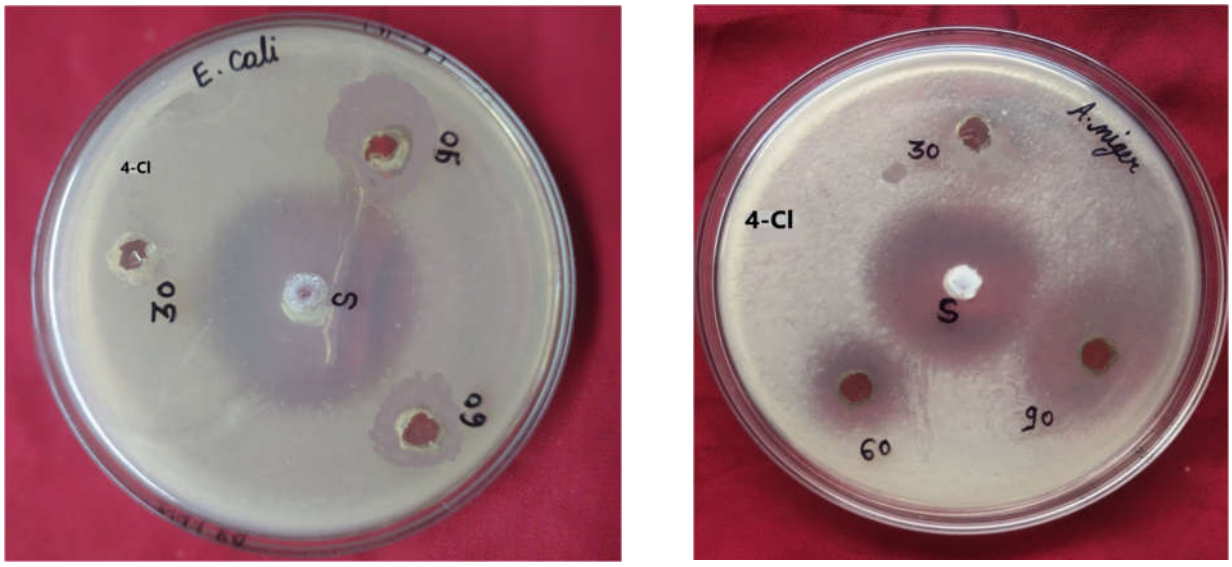


Figure3: Antibacterial and Antifungal activity of 4a (Chloro derivative).

6. Biological Screening

The synthesized pyrazole derivatives were evaluated for antimicrobial activity against *Escherichia coli* (bacteria) and *Aspergillus niger* (fungi) using the agar well diffusion method. Antimicrobial zones were recorded at three concentrations — 30 µM, 60 µM, and 90 µM — to determine dose-dependent behavior.

Table 3. Antibacterial Activity Against E. coli (Zone of Inhibition in mm)

Compound	Substituent (R)	30 µM	60 µM	90 µM
4a	4-Cl	12	15	18
4b	3-NO_2	11	14	17
4c	4-NO_2	13	17	19
4d	4-OH	9	12	15
4e	4-OCH_3	10	13	16
4f	H	8	10	13
STD	Streptomycin	19	21	22

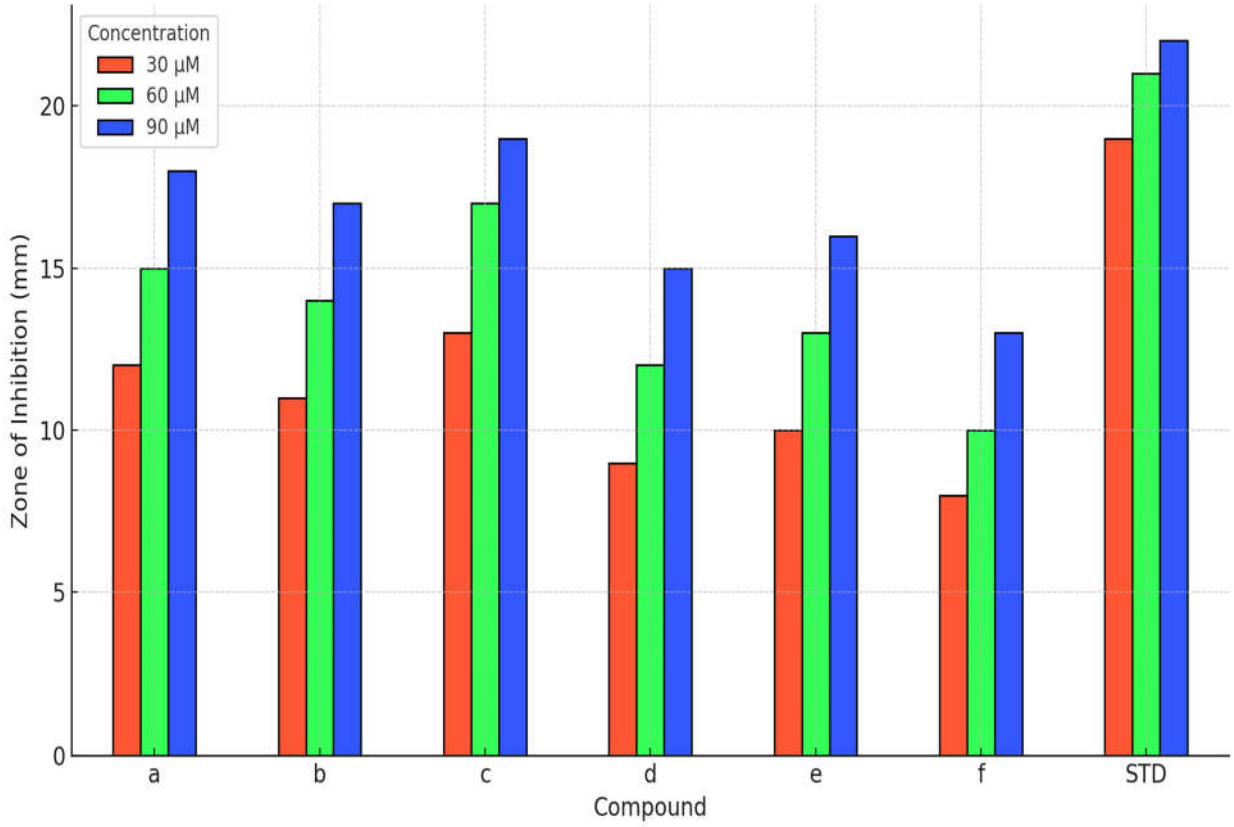


Figure 4: Comparative E.coli antibacterial activity at variable concentrations

Table 4. Antifungal Activity Against A. niger (Zone of Inhibition in mm)

Compound	Substituent (R)	30 µM	60 µM	90 µM
4a	4-Cl	14	17	20
4b	3-NO_2	13	16	18
4c	4-NO_2	15	19	22
4d	4-OH	11	14	17
4e	4-OCH_3	12	15	19
4f	H	10	12	14
STD	Amphotericin B	22	24	25

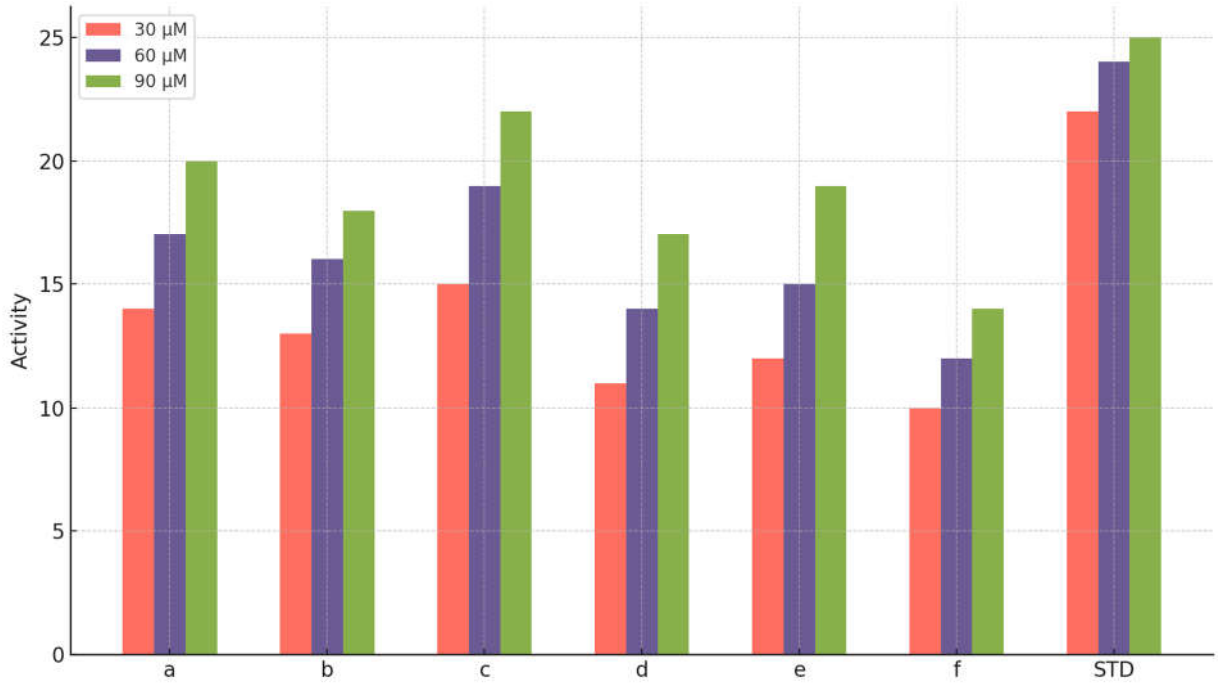


Figure 5: Comparative A.niger antifungal activity at variable concentrations

Comparative Analysis and SAR

- Compound (c) (4-NO_2) emerged as the most potent candidate across both microbial strains and all concentrations, confirming that strong electron-withdrawing groups at the para position significantly enhance activity.
- Compound (a) (4-Cl) also demonstrated excellent activity, especially against A. niger, owing to the halogen's role in increasing lipophilicity and membrane permeability[20].
- Compounds (d) and (e) (with –OH and –OCH_3 groups) showed moderate but consistent activity, reflecting weaker electronic effects.
- A clear concentration-dependent trend was observed, confirming that these molecules act in a dose-responsive manner.

7. Conclusion

A simple, efficient, and green synthetic protocol was developed for the preparation of novel pyrazole derivatives under solvent-free grinding conditions. Compared to traditional methods, the green mechano-chemical grinding synthesis demonstrated significant advantages including enhanced yields, faster reactions, and improved environmental safety. Biological screening revealed potent antibacterial and antifungal activities, particularly for nitro-substituted derivatives. These findings highlight the promising therapeutic potential of pyrazole compounds and the importance of sustainable methodologies in drug development.

8. References

[1] P. C. Lv, H. Q. Li, J. Sun, Y. Zhou, and H. L. Zhu, "Synthesis and biological evaluation of pyrazole derivatives containing thiourea skeleton as anticancer agents," *Bioorg. Med. Chem.*, vol. 18, no. 13, pp. 4606–4614, 2010.

[2] Ş. G. Küçükgüzel and S. Şenkardeş, "Recent advances in bioactive pyrazoles," *Eur. J. Med. Chem.*, vol. 97, pp. 786–815, 2015.

[3] S. Sathish Kumar and H. P. Kavitha, "Synthesis and biological applications of triazole derivatives — a review," *Mini-Rev. Org. Chem.*, vol. 10, no. 1, pp. 40–65, 2013.

[4] L. M. Aroua, F. M. Alminderej, H. R. Almuhaylan, A. H. Alosaimi, F. Medini, H. A. Mohammed, et al., "Benzimidazole(s): synthons, bioactive lead structures, total synthesis, and the profiling of major bioactive categories," *RSC Adv.*, vol. 15, no. 10, pp. 7571–7608, 2025.

[5] M. A. Alam, "Pyrazole: An emerging privileged scaffold in drug discovery," *Future Med. Chem.*, vol. 15, no. 21, pp. 2011–2023, 2023.

[6] K. Karrouchi, S. Radi, Y. Ramli, J. Taoufik, Y. N. Mabkhot, F. A. Al-Aizari, and M. H. Ansar, "Synthesis and pharmacological activities of pyrazole derivatives: A review," *Molecules*, vol. 23, no. 1, Art. no. 134, 2018.

[7] C. J. Clarke, W. C. Tu, O. Levers, A. Brohl, and J. P. Hallett, "Green and sustainable solvents in chemical processes," *Chem. Rev.*, vol. 118, no. 2, pp. 747–800, 2018.

[8] P. Anastas and N. Eghbali, "Green chemistry: principles and practice," *Chem. Soc. Rev.*, vol. 39, no. 1, pp. 301–312, 2010.

[9] D. Dharmendra, P. Chundawat, Y. Vyas, P. Chaubisa, and C. Ameta, "Greener design and characterization of biochar/Fe_3O_4@SiO_2–Ag magnetic nanocomposite as efficient catalyst for synthesis of bioactive benzylpyrazolyl coumarin derivatives," *RSC Adv.*, vol. 13, no. 21, pp. 14594–14613, 2023.

[10] D. Tan and F. García, "Main group mechanochemistry: from curiosity to established protocols," *Chem. Soc. Rev.*, vol. 48, no. 8, pp. 2274–2292, 2019.

[11] R. Ratti, "Industrial applications of green chemistry: Status, challenges and prospects," *SN Appl. Sci.*, vol. 2, no. 2, Art. no. 263, 2020.

[12] S. Mor, M. Khatri, R. Punia, S. Nagoria, and S. Sindhu, "A new insight into the synthesis and biological activities of pyrazole-based derivatives," *Mini-Rev. Org. Chem.*, vol. 19, no. 6, pp. 717–778, 2022.

[13] P. Horrocks, M. R. Pickard, H. H. Parekh, S. P. Patel, and R. B. Pathak, "Synthesis and biological evaluation of 3-(4-chlorophenyl)-4-substituted pyrazole derivatives," *Org. Biomol. Chem.*, vol. 11, no. 29, pp. 4891–4898, 2013.

[14] V. Rastija, K. Vrandečić, J. Ćosić, G. Kanižai Šarić, I. Majić, D. Agić, et al., "Antifungal activities of fluorinated pyrazole aldehydes on phytopathogenic fungi, and their effect on entomopathogenic nematodes, and soil-beneficial bacteria," *Int. J. Mol. Sci.*, vol. 24, no. 11, Art. no. 9335, 2023.

[15] M. U. Rehman, F. He, X. Shu, J. Guo, Z. Liu, S. Cao, and S. Long, "Antibacterial and antifungal pyrazoles based on different construction strategies," *Eur. J. Med. Chem.*, Art. no. 117081, 2024.

[16] S. B. Guo, S. X. Wang, and J. T. Li, “D,L-proline-catalyzed one-pot synthesis of pyrans and pyrano[2,3-c]pyrazole derivatives by a grinding method under solvent-free conditions,” *Synth. Commun.*, vol. 37, no. 13, pp. 2111–2120, 2007.

[17] M. Leonardi, M. Villacampa, and J. C. Menéndez, “Multicomponent mechanochemical synthesis,” *Chem. Sci.*, vol. 9, no. 8, pp. 2042–2064, 2018.

[18] K. Tanaka, *Solvent-free Organic Synthesis*. John Wiley & Sons, 2009.

[19] A. Ahmad, S. Rao, and N. S. Shetty, “Green multicomponent synthesis of pyrano[2,3-c]pyrazole derivatives: current insights and future directions,” *RSC Adv.*, vol. 13, no. 41, pp. 28798–28833, 2023.

[20] B. R. Naidu, T. Sruthi, R. Mitty, and K. Venkateswarlu, “Catalyst-free mechanochemistry as a versatile tool in synthetic chemistry: a review,” *Green Chem.*, vol. 25, no. 16, pp. 6120–6148, 2023.