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Microwave Assisted Synthesis and Biological Screening of Mannich Bases

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KEYWORDS

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Aromatic amines,
Anthelmintic activity,
Microwave,
Pheretima posthuma

ABSTRACT:

The microwave-assisted synthesis of 3-methyl 5-pyrazolone was performed by using ethyl acetoacetate and hydrazine hydrate. It was then reacted with different aromatic amines under microwave irradiation to obtain different Mannich bases. The synthesized compounds were characterized by MP, TLC, & FT-IR. All synthesized compounds were screened for anthelmintic activity using *Pheretima posthuma* (Indian earthworm) Time of paralysis (TOP) and Time of death (TOD) were reported. Albendazole was used as a standard. Synthesized compounds showed moderate to good activity.

1. Introduction

The Mannich reaction is a three-component organic reaction involving the amino alkylation of an acidic proton adjacent to a carbonyl functional group by formaldehyde and a primary or secondary amine or ammonia. The final product is a β-amino-carbonyl compound, also known as a Mannich base. Named after Carl Mannich, the reaction begins with the nucleophilic addition of an amine to a carbonyl group, followed by dehydration to form a Schiff base. This Schiff base, acting as an electrophile, then undergoes electrophilic addition with an enol derived from a carbonyl compound containing an acidic α-proton.[1] In the Mannich reaction, primary or secondary amines react with formaldehyde to form a Schiff base. Tertiary amines, lacking an N-H proton, do not participate in this reaction. The Schiff base can then react with acidic compounds (nucleophiles), including carbonyl compounds, nitriles, acetylenes, and aliphatic nitro compounds. Additionally, activated phenyl groups and electron-rich heterocycles such as furan, pyrrole, and thiophene can be used. The Mannich reaction can be viewed as involving a mixed-aldol reaction, followed by the dehydration of the alcohol and the conjugate addition of an amine (Michael reaction).[2]

Previous research on Mannich reaction has been examined, with details drawn from various sources. Selvam *et al*. (2012) reported a series of 1-(4-substituted phenyl)-3-phenyl-1H-pyrazole-4-carbaldehydes under microwave irradiation. Formation of the pyrazole derivatives was achieved by treating with Vilsmeier-Haack reagent. The newly synthesized compounds were evaluated for their anti-inflammatory and analgesic activities compared to Diclofenac sodium as a standard drug.[3] Bule *et al.* (2013) have reported the synthesis and biological evaluation of newly synthesized pyrazolone derivatives for COX-2 inhibitory activities.[4] Dube *et al.* (2015) have performed the synthesis of 3-methyl-5-pyrazolone derivatives and evaluated their in-vitro cytotoxic activity by two Models of shrimp lethality bioassay and SEB assay.[5] Sravanthi *et al.* (2017) have synthesized a series of novel pyrazole, triazole-based benzo hydrazones via conventional and microwave methods in the presence of an acetic acid catalyst. The microwave method provided a green and economical approach to the synthesis of novel Schiff bases. Some

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intermediates and all the final compounds were characterized by NMR, mass, and elemental analysis. The compounds were screened for their in vitro antibacterial activity against Gram-negative bacteria (*Escherichia coli & Pseudomonas Aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*).[6] Marco *et al.* (2009) A rapid protocol for the multicomponent microwave-assisted organocatalytic domino Knoevenagel/hetero Diels–Alder reaction (DKHDA) has been developed for the synthesis of 2,3-dihydropyran[2,3-c] pyrazoles. The compounds were screened for their antibacterial activities.[7]

Sivakumar *et al.* (2014) synthesized some Mannich bases of 5-methyl-2-[(2-oxo-2H-chromen-3-yl) carbonyl]-2,4-dihydro-3H-pyrazol-3-one. And described by using conventional and non-conventional (Microwave) techniques. Microwave-assisted reactions showed that require shorter reaction times and good yield. The newly synthesized compounds were screened for their anti-inflammatory, analgesic activity, antioxidant, and antibacterial effects compared with ciprofloxacin as a standard drug.[8] Marcos *et al.* (2003) synthesized a series of 5-trichloromethyl-1-phenyl-1H-pyrazoles and 5-trichloromethyl-1,2-dimethylpyrazolium chloride under microwave irradiation. The formation of pyrazole derivatives was achieved by treating with phenylhydrazine and 1,2-dimethylhydrazine dihydrochloride, respectively, using toluene as solvent.[9] Zhang *et al.* (2016) have synthesized 4-substituted pyrazolone derivatives developed via condensation starting from ethyl acetoacetate, hydrazine, and isoxazole-aldehyde over solid support SiO_2 under microwave-assisted solvent-free conditions in satisfactory yields.[10] Aljuhani *et al.* (2021) have synthesized copper oxide-chitosan nanocomposite via a simple casting method which was characterized by different analytical techniques, FTIR, X-ray diffraction, and thermogravimetric analysis. Chitosan nanocomposite has proven to be an excellent heterogeneous base catalyst to give 1,3,4-trisubstituted pyrazoles under microwave irradiation.[11]

Filho *et al.* (2021) have reported synthesis, docking, machine learning, and antiproliferative activity of the 6-ferrocene/heterocycle-2-aminopyrimidine and 5-ferrocene-1H-Pyrazole derivatives obtained by microwave-assisted Atwal reaction.[12] Paul *et al.* (2001) have synthesized pyrazole [3,4-b] quinolines and pyrazole [3,4-c] pyrazoles have been synthesized from β-chlorovinylaldehydes and hydrazine hydrate/phenylhydrazine using p-TsOH under microwave irradiation.[13] Patricia *et al.* (2008) have reported the synthesis and evaluation of the analgesic and anti-inflammatory properties of novel 3- or 4-substituted 5-trifluoromethyl-5-hydroxy-4,5-dihydro-1H-1-carboxyamidepyrazoles.[14] Coree *et al.* (2014) have reported the synthesis of 4-amino-3-cyano-N-arylpyrazoles based on a Thorpe–Ziegler cyclization using microwave activation, via a new diversity-oriented synthetic pathway, these highly functionalized building blocks allowed access to various heteroaromatic scaffolds such as Pyra-zolo-pyridines, pyrazole-pyrimidine and pyrazole-oxadiazoles.[15] Polshettiwar *et al.* (2014) have reported the nano-organocatalyst: magnetically retrievable ferrite-anchored glutathione by microwave-assisted Paal–Knorr reaction.[16] Althagafi *et al.* (2017) have reported the microwave-assisted regioselective synthesis of novel pyrazoles and pyrazolopyridazine via fluorine-containing building blocks.[17]

This research aims to perform microwave-assisted synthesis of Mannich bases, the newly synthesized compound will be characterized by determining its melting point (MP), conducting thin-layer chromatography (TLC), and performing spectral characterization using Fourier-transform infrared spectroscopy (FT-IR). Following this, the anthelmintic activity of the newly synthesized compound will be evaluated using a standard protocol.

2. Materials

2.1 Chemical and reagents

In this study, the Indian earthworm species (*Pheretima posthuma*) was obtained from local farmers, Akole, Maharashtra, India. Marketed formulations of Albendazole suspension and saline water were purchased from a local pharmacy shop. Finar Ltd. supplied purified water, and ethanol in Ahmedabad, Gujarat, India. Likewise, analytical-grade p-nitroaniline, O-nitroaniline, hydrazine hydrate, and formaldehyde were provided by Merck Ltd. in Mumbai, India.

Journal of Chemical Health Risks

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2.2 Instrumentation

Bajaj MTBX oven was employed for the entire microwave-assisted synthesis reaction, Although, an electronic balance, centrifuge, and vortex mixer were used.

3. Experimental Work

3.1 Procedure for synthesis of 3-methyl pyrazole-5-one

Ethyl acetoacetate 0.05 mole (6.5g) and hydrazine hydrate 0.05 mole (5.4g) were mixed in an open beaker (100ml) and then the mixture was irradiated in a microwave oven (280W) for 30 seconds to 2 minutes. After cooling, ether (30ml) was added, and the separated crystals were filtered. The product was recrystallized in a mixture of water and ethanol (1:1) to obtain white crystals. The resulting reactions are shown in Figure 1.

3.2 Procedure for synthesis of 5-Methyl-4-phenylamino methyl-2,4-dihydro-pyrazole-3-one

To a solution of the compound 5-methyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (0.01 mol) in ethanol (30ml), formaldehyde (0.02 mol) and the corresponding substituted amines (0.01 mol) were added and the mixture was kept in a microwave oven for 30 seconds to 2 minutes. It was then poured into ice water, and the precipitated solid was filtered off, dried, and recrystallized from ethanol. The resulting reactions are shown in Figure 2.

Scheme-1

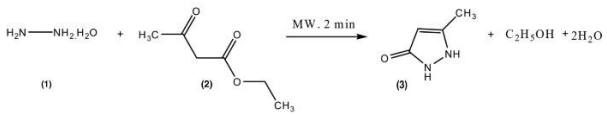
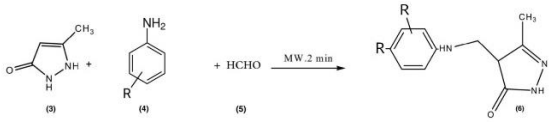


Fig. 1. Synthesis reaction of 3-methyl pyrazole-5-one

Scheme-2



3446.79 cm-1	NH Stretching
1595.13 cm-1,1442.75 cm-1	C=C Stretching
1313.52 cm-1	C-N Stretching

Fig. 2. Synthesis of 5-Methyl-4-phenylamino methyl-2,4-dihydro-pyrazole-3-one

4. Results And Discussion

The Mannich bases were synthesized using different aromatic amines and formaldehyde. The reactions are shown in Figures 1 and 2. Furthermore, the physicochemical characterization of the synthesized compounds (6a-c) was carried out using thin-layer chromatography and melting point analysis. The obtained results are illustrated in Table 1. To authenticate the synthesized compounds, the FT-IR spectra were taken. The obtained spectra and their interpretations for compounds (6a-c) are shown in Figures 3, 4, and 5, and tabulated in Tables 2, 3, and 4, respectively.

Table 1. Physicochemical characteristics of synthesized compounds (6a-c)

Sr. no	Compound Code	-R	Mol. Formula	Mol. Weight	RF Value	% Yield	Melting Point
1	6a	-H	$C_{11}H_{13}N_3O$	203.21	0.50	81.51	112-116 ^{O}C
2	6b	p-NO_2	$C_{11}H_{13}N_4O_3$	249.22	0.46	85.13	122-126 ^{O}C
3	6c	o-NO_2	$C_{11}H_{13}N_4O_3$	249.22	0.77	60.50	132-136 ^{O}C

TLC- n-Hexane: Ethyl acetate (9:1)

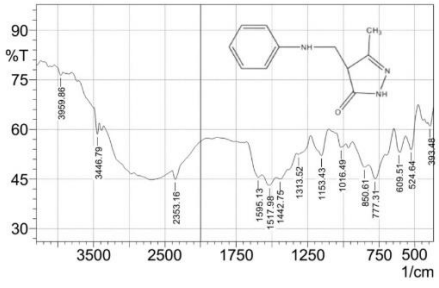


Fig. 3. FT-IR spectrum of compound 6a

Journal of Chemical Health Risks
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Table 2. Interpretation of FT-IR spectrum of compound 6a

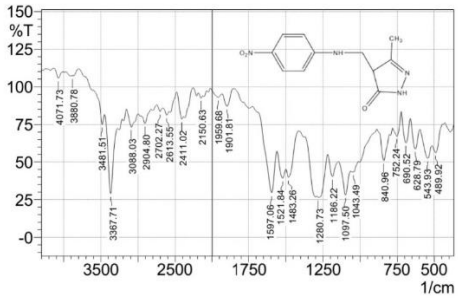

Fig. 4. FT-IR spectrum of compound 6b

Table 3. Interpretation of FT-IR spectrum of compound 6b

3367.71 cm-¹	NH Stretching
3088.03 cm-¹	Sp^2 CH Stretching
2904.80 cm-¹	Sp^3 CH Stretching
1597.06 cm-¹, 1483.26 cm-¹	C=C Stretching
1521.84 cm¹, 1280.73 cm-¹	C-NO_2 Stretching

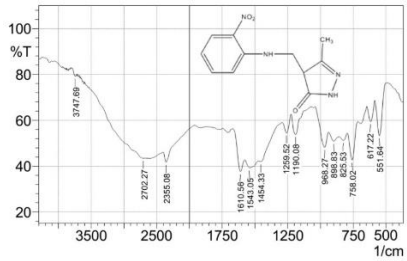

Fig. 5. FT-IR spectrum of compound 6c

Table 4. Interpretation of FT-IR spectrum of compound 6c

3747.69 cm-¹	NH Stretching
1543.05 cm-¹, 1259.52 cm-¹	C-NO_2 Stretching
1610.56 cm-¹, 1454.33 cm-¹	C=C Stretching
2702.27 cm-¹	CH Stretching
1190.08 cm-¹	C-N Stretching

Additionally, the synthesized compounds were tested for anthelmintic activity using *Pheretima posthuma* (Indian Earthworm). The time of paralysis (TOP) and time of death (TOD) were calculated. Albendazole was selected as the standard; the TOP was observed to be 34 minutes for 10 ppm and 14 minutes for 20 ppm, whereas TOD was found to be 55 minutes for 10 ppm and 40 minutes for 20 ppm, as shown in Figure 6. Among all the synthesized compounds, compound 6b showed moderate to good activity. For compound 6b, the TOP was observed to be 52 minutes for 10 ppm and 35 minutes for 20 ppm, whereas the TOD was observed to be 68 minutes for 10 ppm and 54 minutes for 20 ppm, as shown in Figure 7. The obtained values are tabulated in Table 5.

Fig. 6. TOP and TOD of *Pheretima posthuma* in albendazole suspension

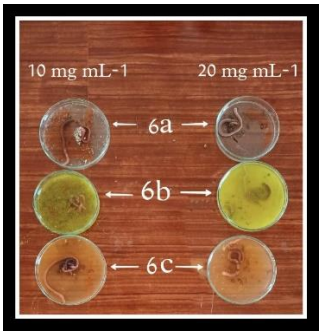

Fig. 7. TOP and TOD of *Pheretima posthuma* in compound 6a-c

Table 5. Results of anthelmintic activity of synthesized compound (6a-c).

Compound Code	**Time of Paralysis (min)**		**Time of Death (min)**	
	10mg/ml	20mg/ml	10mg/ml	20mg/ml
6a	58	45	84	74
6b	**52**	**35**	**68**	**54**
6c	65	52	90	78
Std. (Albendazole Suspension)	34	14	55	40

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5. Conclusion

The Mannich bases were synthesized, characterized, and screened for anthelmintic activity using *Pherethima posthuma* (Indian earthworm). Synthesized compound 6b showed moderate to good activity.

6. Future Scope

The compounds were synthesized by microwave irradiation. The yield was found to be higher as compared to conventional synthesis. The synthesized compounds were screened for anthelmintic activity using *Pherethima posthuma* (Indian earthworm). Synthesized compounds 6b showed good activity. With suitable modification, these compounds can be tested for various biological activities.

7. Acknowledgment

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8. Conflict Of Interest

All authors report that they have no conflict of interest in any financial and personal relationships with other people or organizations that could have appeared to influence the work reported in this paper.

9. Funding Sources

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10. Ethics Approval Statement

No study involves an experiment on humans and animals.

11. Data Availability Statement

Not Applicable

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Journal of Chemical Health Risks

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