Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2022.v10.i01.A03

ECO-FRIENDLY MICROWAVE SYNTHESIS OF SUBSTITUTED PYRAZOLE DERIVATIVES AND THEIR MICROBIAL EXPLORATION

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ABSTRACT

A Rapid and simple procedure free from exiting protocol with avoid of toxic, costly catalyst. This catalyst free green synthetic reaction as a retrievable and utility green solvent in reaction fashion clearly displayed a high attraction in the vicinity of green approach is efficient step to improved atomic economic for the synthesis of pyrazoles derivatives in microwave method via multi-component one pot synthesis from 1, 3 diketones with hydrazine hydrates in glycerol solvent media this modern strategy could serve as a valuable synthetic alternative for green synthesis reactions process diverge in terms of economic aspects and pharmaceutical profiles of novel pyrazoles derivatives. Chemical Structures confirmed by FT-IR, Proton and Carbon NMR spectral data. We investigate *In vitro* antibacterial activity of synthesized compounds against, *St.aureus*, *Streptococci, E-coli* etc.

KEYWORDS

Pyrazoles derivatives, Glycerol, Microwave, *In-vitro* screening and Antibacterial activity.

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INTRODUCTION

As analogues of purine based heterocycles is pyrazoles have attract budding synthetic organic pharmacist and druggist attributable one-another biological significance and therapeutic benefit including analgesic[1], antipyretic[2], anticancer[3], antiviral[4], anti-inflammatory[5], antioxidants[6], antimicrobial[7], anti-diabetic[8], anticonvulsant[9], anti-arrhythmic activities[10], anti-tumour activity[10], antitrypanosomal[11], hypnotic[12], anti-tumor[13], antimycobacterial[14], antischistosomal[15], Pyrazole is a multipurpose utility leads to new ideology implementation towards drug investigation by synthetic chemists which biologically efficient and

effective lifesaving molecule. Most of the synthetic pharmaceutical chemistry ideas are differ to the development new preparative method pyrazole moiety scheme which containing reactions to afford a synthesis of novel pyrazole molecule by provides enormous opportunity in the field of modern medicinal chemistry[16]. The existing literature knowledge on research which provide information only about the synthetic strategy of pyrazoles and their outcomes during the recent year and note about little importance to biological activities [17].Recent synthetic organic chemistry literature survey gave i.e. Zaleplon used as an ideal drug forhypnotism which was clearly mimic the structure of pyrazoles and also attracts a lot of curiosity of pyrazoles derivatives[18]. In the literature clearly reported i.e. core pyrazoles not only shows remarkable activity and also their moieties shows drastic biological graphs hence pyrazoles of great scientific interest recent days they are widely found in bioorganic chemistry as well as synthetic chemistry and medicinal chemistry for new drug discovery[19]. They also found application in industrial production of antioxidants receives more and more attention due to unique qualities and their application as anticancer agent[20]. Substituted pyrazoles scaffolds of privileges to pyrazoles backbone in synthetic pharmacology report cytotoxic of cell cancer[21]. It clearly emphasized that different proportion of hydrazine ring pyrazoles with another substituted heterocycles is facilitated arise of new strategy to designing substituted drug molecules which implements special achievement in pharmacological profile by the action of toxicity lower ideology[22].

Substituted pyrazoles are novel class of potent and selective anticancer agent and display characteristic profiles of cytotoxic response across the cell membrane. Annulated ring substitution to the Pyrazolones rings, likely hydrazine annulated methyl pyrazolo[23], aminodihydropyrrolo[24], phenylpyrazoles[25] and they also clearly show diverse biological spectrum. However, the synthetic and biological profile of substituted hydrazine hydrate moieties of pyrazoles too limited in number and they are suffering from some demerits including utility of toxic reagents, high volatile solvent, expensive catalyst, harsh reaction condition. Avoiding previous tedious synthetic protocols. Therefore, development of efficient synthetic plan of hydrazine annulated pyrazoles and pyrazoles under microwave conditions is an environment friendly methods needed to implement.

EXPERIMENTAL DETAILS

Chemicals and apparatus

Commonly selected chemicals are analytical grade used for preparation. MP are determined by Bucchi-450 melting point apparatus. Infrared spectra were reported using KBr pellets on BRUKER-FTIR spectrometry. The ^{1}H (proton) NMR notes (300 MHz) and ^{13}C (carbon) NMR notes (75 MHz) spectra recorded in a Varian XL300 spectrometry. chemical shifts (j) were record in ppm relatives to TMS (tetramethylsilane), and multiple peaks are named as s singlet (s), broad singlet (bs), doublet (d), triplet (t), quartet (q), and multiplet (m). Usually reactions progress was monitored with TLC [Thin layer chromatography] carried out onsilica gel (Merck) plates (pre-coated), Spots are detect using (254 and 366nm) in UV chamber.

Synthetic methodology of substituted-pyrazoles

In recent investigation focused on preparation of substituted-pyrazole moiety which shows significant pharma logical displaying a double multitude utility in field of pharmaceutical, medicinal profile of substituted derivatives improperly increases the synthetic ideology which clearly differ from other the addition small substitution group, structure, ring will greatly alter the biological importance's. Consequently, our new synthetic approaches for the synthesis substituted pyrazoles by the expansion of green methods for effective construction of substituted pyrazole analogs are the main aim of this article by the implementation of green, and eco-friendly green solvent synthetic approach for preparation substituted like pyrazole and pyrazolono-pyrazoles analogues are main context. 3.1 Green synthetic ideology utility of Microwave irradiation via green solvent: An innovative pollution free strategy for the creation of substituted pyrazoles from hydrazine

hydrate (α, β unsaturated diketones) have β -hydrogen with glycerol (Scheme-01). In this new observation, reaction findings were brought about in microwave mediated reaction (MWR) with the proposed plan based green-solvent procedure, outcome expectation in terms of excellent product and very minute reaction time were clearly observed, without any intermediate which are indirectly influence this ideology as environment pollution free, rapid reaction synthetic route to attain substituted-pyrazoles using unsaturated diketones which have β -hydrogen.

Preparation of substituted Pyrazolones by conventional methods

Analytical grade chemicals are selected for the preparation of substituted Pyrazolones by the hydrazine hydrate ($NHNH_2H_2O$) (5m.ml) were slowly transfer to the subsequent α, β -diketones (5m.ml) to the RBF and reaction mixture thoroughly mix well kept for stirring for few minutes by adding magnetic bit adjusting 525rpm magnetic stirrer at normal temperature to get homogeneous mixture. Then slowly adding glycerol approximately (8m.ml), under vigorous stirring, continue for 40-45 min. Reaction progress was monitored by TLC (Thin layer chromatography) by using pet- ether and ethyl acetate in the ration 7:3 respectively. Chemical spots were noted by iodine /UV chamber. By this process cotton precipitate was obtained. Cool it for few minute then slowly transfer to ice-cold water and filter off preliminarily washed with distilled water many time followed by acetone/ETOH and dried for 5-6 hrs at 65 ^{0}Cunder Vacuum. Obtained pure substituted pyrazoles derivatives (scheme-3a-g). Same protocol to be continued for different 1, 3 diketones (3b) reacted with (2a) hydrazine hydrate to give substituted-pyrazole (3c) yield achievement up to (40-96%). Replacing the aryls group by hetero aryls. the cyclo-condensation complete in 40-45 min with hydrazine hydrates in presence of glycerol we confirm i.e. conventional condition also efficiently to carried out reaction forming substituted-pyrazole achieving yield about 40-96%. Recovery of the solvent by the elimination of the acetone/ EtOH, it can be reused further.

Preparation substituted Pyrazolones by microwave methods

The substituted Pyrazolones by hydrazine hydrate ($NHNH_2H_2O$) (0.5m.ml) were slowly transfer to the subsequent α, β -diketones (0.5m.ml) taken in RBF run for few minutes for homogeneous fixing of diketones, then addition of glycerol (8 ml) to the reaction mixture mix well thoroughly by vigorous stirring and mixture irradiated in microwave at normal temperature continue for 8-10 min. reaction progress was monitored by TLC technique (Thin layer chromatography) by using solvent ratio (Pet.ether: ethyl acetate 7:3), chemical spots were noted by iodine /UV chamber. Cotton precipitate was formed for about 8-10 min. Then transfer to ice cold water gently stirred few minutes and superannuated remove then solid filter off, purified washed with distilled water many time followed by acetone/ETOH and dried for 5-6 hrs at 65^{o}C under Vacuum. Obtained pure substituted pyrazoles derivatives (scheme-3a-g). Same protocol to be continued for different 1, 3 diketones (3b) reacted with (2a) hydrazine hydrate to give substituted-pyrazole (3c) yield achievement up to (83-86%). Replacing the aryls group by hetero aryls. the cyclo-condensation complete in 8-19 min with hydrazine hydrates in presence of glycerol green solvent we confirm i.e. Microwave irradiation condition is more efficient than conventional step carried out and forming substituted-pyrazole achieving yield about 83-86%. Recovery of the solvent by the elimination of the EtOH, it can be reused further.

Selected spectral data

1, 3, 5-triphenyl-2, and 3-dihydro-1*H*-pyrazole: (3d): Melting Point

138-139°C, FT-IR (*KBr-Pellet*) Cm^{-1}: 3397(N-H Stretch, Pyrazole ring), 3073, 3028 (=C-H, hydrazine substituent), 1875 9 (C-H bending, Phenyl), 1263 9 (C-N bending, Pyrazole ring). *^{1}H NMR data* (DMSO-d_6)- δ 2.45 singlet, (3H, Ar-CH_3), 4.82 base singlet, (2H, -NH_2), *^{13}C NMR data* (DMSO-d6): δ 23.4, 76.2, 87.5, 115.4, 147.3, 150.2, 151.5, 162.3.

1-[(3*Z*)-5 methyl 2, 4 dihydro-3 *H*-pyrazole 3ylidene]: (3e): Melting Point

160-162°C, FT-IR (*KBr-Pellet*): Cm^{-1}3594 (N H stretch, Pyrazole ring), 3389 (NH_2, amine),1772 (C=S stretch), 1676 (C=N medium, Pyrazole ring).*^{1}H NMR data* (DMSO-d_6) δ 2.45 singlet, (3H, Ar-CH_3), 4.82 base singlet, (2H, -NH2), *^{13}C NMR data* (DMSO-d_6): δ 25.4, 76.2, 87.5, 115.4, 147.3, 150.2, 150.5, 162.3.

1 [(3*Z*) 5 methyl-2, 4-dihydro-3 *H* pyrazol-3-ylidene]: (3f): Melting Point

140-142°C, IR *(KBr-Pellet*): Cm^{-1} 3438 (N-H stretch, Pyrazole ring), 3378 (NH_2, amine), 2174 (C=N stretch, Pyrazole ring) 1616.*^{1}H NMR data* (DMSO-d_6): δ 2.45 singlet, (3H, Ar-CH3), 4.82 base single, (2H, -NH2), *^{13}C NMR data* (DMSO-d_6): δ 23.4, 76.2, 88.5, 113.4, 148.3, 148.2, 152.5, 161.3.

RESULTS AND DISCUSSION

4.1 altering the chemical backbones structures by substituted groups is our main aim of investment selected part of on-going new protocol investigations, here we directed research knowledge towards the comparison between conventional and microwave irradiation method by the comparative study give information about microwave methodology is too efficient in terms of reaction time and yield. We focus on literature survey on synthesis of substituted pyrazoles derivatives using (1) *α, β-unsaturated diketones* as a substrate. (3a) and in presence of different eco-friendly solvent. All the reactions are conducted in zero-degree temperature in microwave irradiation method. Compounds 1-5 were prepared by the recent literature protocols. (3b) the synthetic reaction series selectively of substituted heterocyclic Pyrazolones scaffolds, 7-hydrazinyl-5-methylpyrazolo (4a) reported in Scheme No.1. According to literature knowledge's present work, reactions which were conducted in different solvents but achieve poor product yield. Present ideology glycerol was used and excellent yield will be achieved. Because solvent have little viscosity and shows spontaneous reactivity, product yield was excellent. Compound (1) and *hydrazine hydrate in Glycerol solvent* under zero-degree temperature gives substituted pyrazoles derivatives. (Scheme No.1-5) in that methylcyanoacetate gives -92.6% yield and acetyl acetone gives -90.0 % yield least yield given by ethylacetoacetate i.e. 78.8.%. Pure and excellent yield compounds are confirmed by spectral characterised. For occurrence, ^{1}H NMR data showed broad singlet peak (chemical shift) at δ 4.82, 9.56 which were confirm -NH_2 and -NH protons, respectively. While the FT-IR spectrum clearly reveal brawny absorption bands at 3591cm^{-1}, 3321 cm^{-1}, 3267cm^{-1} and 2219 cm^{-1} which designates and recommended the existence of -NH_2, -NH and –CN, respectively.

Microbial Activity

The bacterial activity was investigating by agar-agar diffusion cup plate method against culture strains of E. coli and St. aureus. Salmonella typhi and *Streptococci*. Against standard drug gentamicin used as testimonial in performing a microbial assay. The synthesized derivation was initiate to be most potent towards microbial activity. The synthesized derivatives also showed drastic potent Antibacterial activity; investigated by nutrient agar cup-plate method for cultural pathogenic species *E. coli, St-aureus* and *Streptococci* and gentamycin as standard drug.

Bacterial activity assay

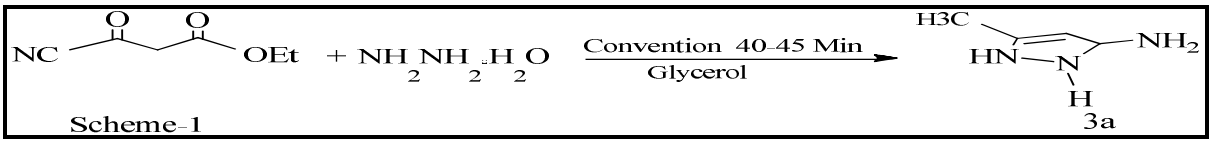
After the of completion of incubation periods. Exact experimental performance done in triplicates. We are fallowed about *in vitro* bacterial activity of synthesized products try out at 1mg/ml Con^n reveal low to high potent against *St. Aureus* inhibition zones of the individual microbial growth can be measured in millimetre scale.

Table No.1: Reaction of substituted pyrazoles derivatives (3a-g) by Microwave method

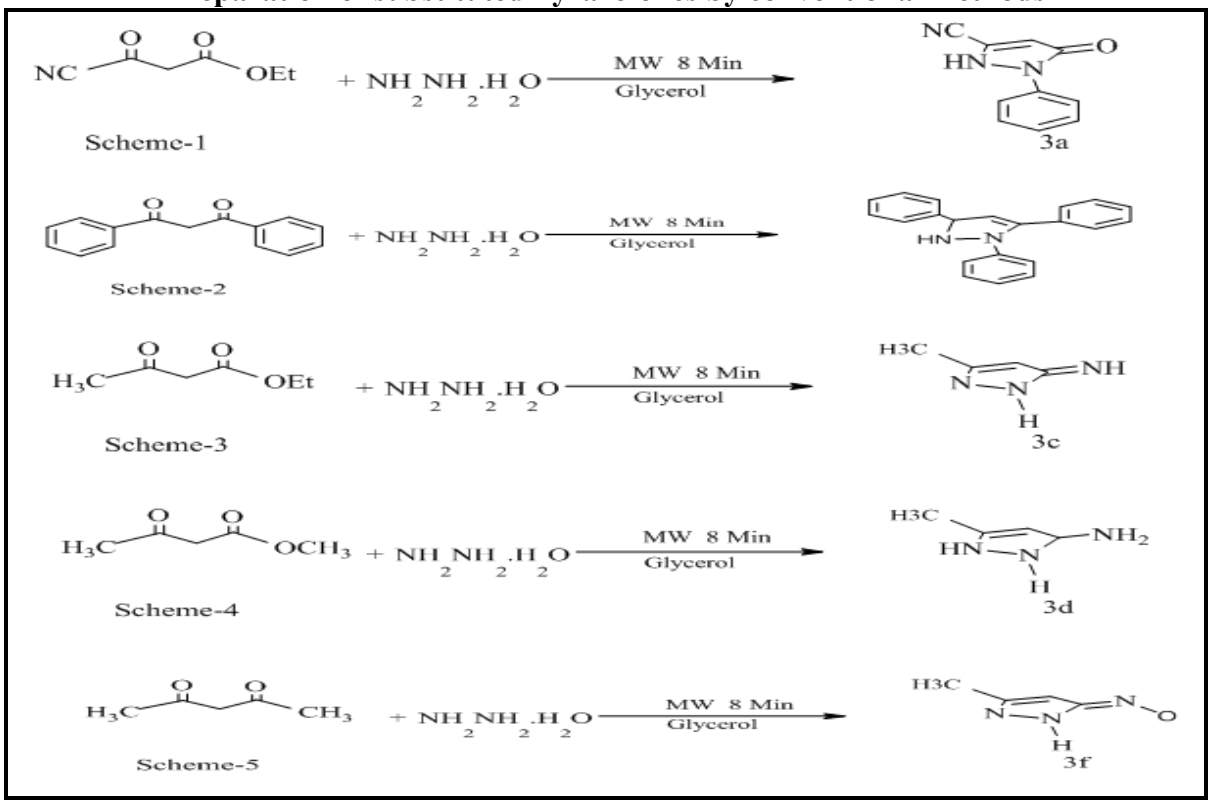
Samples	Different diketones	Conventional stirring		Microwave irradiation		M.P °C
		Without Solvent	Yield in (%)	With Solvent	Yield in (%)	
3a	Ethylcyanoacetate.	45 Min	43.2	8 Min	82.6	273 °C
3b	Benzoin.	40 Min	40.4	8 Min	86.3	128 °C
3c	Methylcyanoacetat	40 Min	42.3	8 Min	92.6	178 °C
3d	Acetyl acetone	42 Min	46.3	8 Min	90.0	139 °C
3e	Methlyacetoacetate	45 Min	45.2	10 Min	84.5	162 °C
3f	Diethylmalonate	40 Min	43.7	8 Min	88.9	142 °C
3g	Ethyl acetoacetate.	40 Min	36.4	8 Min	78.8	116 °C

Table No.2: *In vitro* bacterial activity

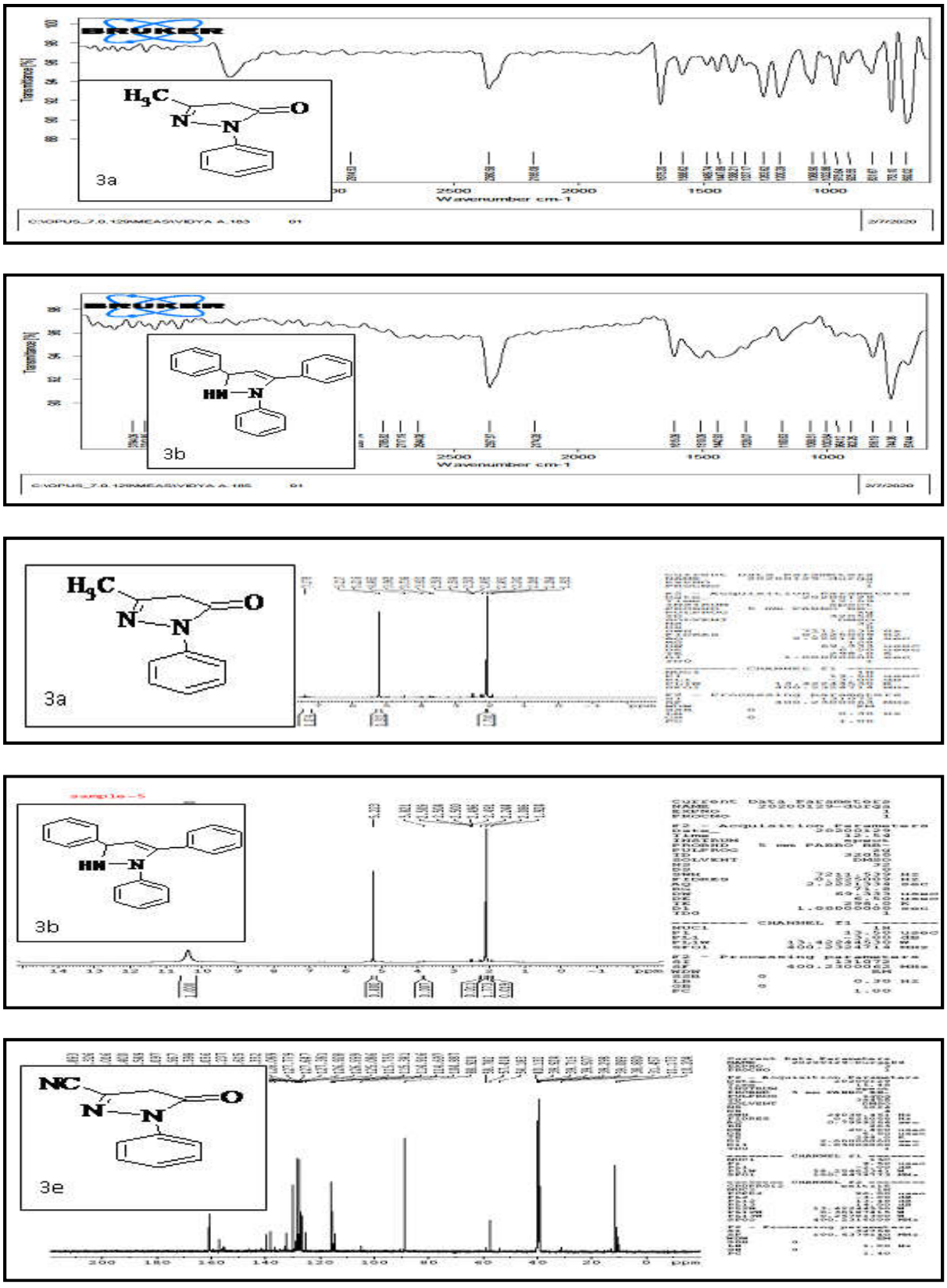
Samples	E-coli	Salmonella typhi	*St. aureus*	Streptococci	Gentamicin
3a	15 ± 0.2	17 ± 0.3	19 ± 0.3	1 7± 0.3	16 ± 0.3
3b	14 ± 0.1	17 ± 0.1	20 ± 0.1	1 6± 0.1	16 ± 0.3
3c	18± 0.2	16 ± 0.9	19 ± 0.3	1 8± 0.2	16 ± 0.3
3d	19 ± 0.2	13 ± 0.3	20 ± 0.2	1 5± 0.0	16 ± 0.3
3e	18 ± 0.2	17 ± 0.2	18 ± 0.2	18 ± 0.2	16 ± 0.3



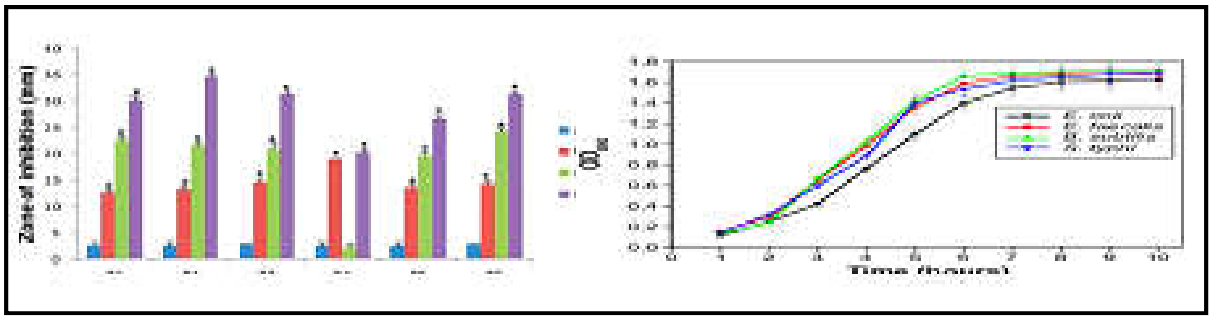
Preparation of substituted Pyrazolones by conventional methods



Schemes No.1: (α, β-unsaturated diketones and hydrazine hydrate in glycerolsolvent)



Selected spectral data of FTIR, 1H NMR and 13C NMR/ (spectral characterization)



CONCLUSION

Our brief interpretation clearly denotes the of glycerol green solvent for countless pharmaceutical operations as moderate medium for drug preparation substituted heterocyclic transformation show improved reactant conversion as product in terms of minutes. Therefore, the synthesized substituted pyrazoles were obtained by both the methods (conventional as well as microwave irradiation) both the methods are operationally simple, but conventional method product yield little less but consume more time. Utility of green solvent improvement atomic economy from expected to excellent yields. This small investigation currently extends with the new aim for creating a small milestone to words substituted pyrazoles. Synthesized products were evaluating for anti-microbial activity. The exploration result initiate that synthesized compounds 3b, 3d are showed high activity 20±0.3 against St-aureus strains and other showed moderate antibacterial activity. Our study reveals that synthesized compounds possessed effective antibacterial activity. MIC (Minimum Inhibitory Concertation) The compound 3b, 3dinhibit St-aureus bacteria in lowest (5.564µg/ml) inhibitory concentration. The remaining compounds i.e. 3a, 3c, 3e, inhibited (6.365µg/ml), (7.455 µg /ml) and (8.265 µg /ml). The compound 3b, 3d inhibited st –aureus at very lowest concertation (5.564 µg/ml) Inhibitory Concertation this conclude newly synthesized compounds (3a-3e) have great potent towards kill or inhibit growth of test bacterial strain in lowest concentration.

ACKNOWLEDGEMENT

Authors sincere thanks to Department of Chemistry, UBDT College of Engineering Davanagere. For providing facility for research work and thanks to Department of chemistry Kuvempu University. For Nano catalyst and thanks to department of Pharmacy SJMIT chitradurga, for FTIR facility and thanks to IISC Bangalore. For NMR facility. Thanks to S.S. Institute of Medical College and Research Centre Davanagere. For providing an opportunity for microbial investigation. Thanks to social welfare Department City Corporation financial support.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Wolfson A. Glycerol: Production, structure and applications; Silva, M.D.S; Ferreira, *Nova Science Publishers, New York,* 2012, 233-248.
2. Calvino-Casilda V. Green solvents I: Properties and applications in chemistry, *Springer: Dordrecht,* 2012, 187-207.
3. Wolfson A, Dlugy C. Glycerol as a green solvent for high product yields and selectivities, *En. Che. Let,* 5(2), 2007, 67-71.
4. Wolfson A, Kimchi H, Tavor D. Nucleophilic substitution of benzyl halides with ammonium acetate in presence of glycerol as green solvent, *As J. Che,* 23(3), 2011, 1227-1229.
5. Wolfson A, Snezhko A, Meyouhas T. Glycerol derivatives as green reaction mediums, *Green Chem. Lett. Rev,* 5(1), 2012, 7-12.

6. Wolfson A, Azran E, Dlugy C, Tavor D. Base catalyzed glycerolysis of benzyl acetate, *Lett. Org. Chem,* 8(7), 2011, 504-508.
7. Kore P P, Kachare S D, Kshirsagar S S, Oswal R J. Base catalyzed glycerolysis of ethyl acetate, *Organic Chem. Curr. Res,* 1(1), 2012, 108.
8. Wolfson A, Naim S, Dlugy C, Tavor D. Acid catalysed transesterification of benzyl acetate in glycerol, *Chim. Oggi,* 30(5), 2012, 69-71.
9. Cabrera D M L, Libero F M, Alves D, Perin G, Lenardao E J, Jacob R G. Glycerol as a recyclable solvent in the microwave-assisted synthesis of disulfides, *Green Chem. Lett. Rev,* 5(3), 2012, 329-336.
10. Thurow S, Webber R, Perin G, Lenardao E J, Alves D. Glycerol/hypo phosphorous acid: An efficient system solvent-reducing agent for the synthesis of 2-organylselanyl pyridines, *Tetrahedron Lett,* 54(24), 2013, 3215-3218.
11. Ying A, Zhang Q, Li H, Shen G, Gong W, He M. An environmentally benign protocol: Catalyst-free Michael addition of aromatic amines to unsaturated ketones in glycerol, *Res. Chem. Intermed,* 39, 2013, 517-525.
12. Bougrin K, Loupy A. Microwave-assisted solvent-free heterocyclic synthesis, *J. Photochem. Photobiol. C, Photochem. Rev,* 6(2-3), 2005, 139-167.
13. Aggarwal V K, De Vicente. A novel one-pot method for the preparation of pyrazoles by 1, 3-dipolar cycloadditions of diazo compounds generated in situ, *J. Org. Chem,* 68(13), 2003, 5381-5383.
14. Kost A N, Grandberg I I. Progress in pyrazole chemistry, *Adv. Heterocycl. Chem,* 6, 1966, 347-429.
15. Padwa A. 1, 3-dipolar cycloaddition chemistry, *John Wiley and Sons, New York,* 1, 1984, 817.
16. Fulton J R, Aggarwal V K, De Vicente J. The use of tosylhydrazone salts as a safe alternative for handling diazo compounds and their applications in organic synthesis, *Eur. J. Org. Chem,* 8, 2005, 1479-1492.
17. Bamford W R, Stevens T S. The decomposition of p-tolylsulfonylhydrazones by alkali, *J. Chem. Soc,* 1952, 4735-4740.
18. Brewbaker J L, Hart H. The cyclization of 3-diazoalkenes to pyrazoles, *J. Am. Chem. Soc,* 91(3), 1969, 711-715.
19. Doyle M P, Yan M J. Effective and highly stereoselective coupling with vinyldiazomethanes to form symmetrical trienes, *J. Org. Chem,* 67(2), 2002, 602-604.
20. Almirante N, Cerri A, Fedrizzi G, Marazzi G, Santagostino M. A general, [1+4] approach to the synthesis of 3(5)-substituted pyrazoles from aldehydes, *Tetrahedron Lett,* 39(20), 1998, 3287-3290.
21. Girish Y R, Kumar K S S, Manasa H S, Shashikanth S. ZnO: An eco-friendly, green nano-catalyst for the synthesis of pyrazole derivatives under aqueous media, *J. Chin. Chem. Soc,* 61(11), 2014, 1175-1179.

Please cite this article in press as: Suresh H M and Suresha Kumara T H. Eco-friendly microwave synthesis of substituted pyrazole derivatives and their microbial exploration, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 10(1), 2022, 13-20.