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Synthesis, Characterization and Antimicrobial Activity of some novel 3,4,5-trisubstituted pyrazolo[3,4-c]pyrazoles

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ABSTRACT

Present work reports the development of a novel, one-pot protocol for the rapid synthesis of pyrazolopyrazoles. The structures of synthesized compounds are in agreement with IR, NMR, and MASS Spectral data. A series of 4,5-disubstituted-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]-pyrazoles (IIIa-f) was prepared by the reaction between 2,4-dinitrophenyl hydrazine, 5-methyl-2,4-dihydro- 3H-pyrazol-3-one (II) and different aldehydes to microwave irradiation. Compound II was obtained by cyclization of ethylacetoacetateate (I) with hydrazine hydrate by stirring in absolute ethanol. Microwaves used to heat the reaction mixture in the step II of the proposed reaction. All the compounds were synthesized with good yield. All the synthesized compounds exhibited antibacterial and antifungal activities at various MIC levels. Compound IIIa showed good activity against Streptococcus Mutans .The zone of inhibition produced by this compound is more than that of standard used for Streptococcus mutans. It shows moderate activity against Staphylococcus aureus, Shigella dysenteriae and Escherichia coli. Compound IIIb showed good activity against Streptococcus mutans and moderate activity against Shigella dysenteriae, Escherichia coli and fungus Candida and Rhizopus. Compound IIId showed moderate activity against Escherichia coli. Compound IIIe showed moderate activity against Streptococcus mutans, Shigella dysenteriae and Rhizopus and good activity against Candida. Compound IIIf showed good activity against Staphylococcus aureus, Candida and Rhizopus and moderate activity against Shigella dysenteriae.

Keywords: Pyrazolopyrazoles, Microwaves, Antibacterial, Antifungal,

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INTRODUCTION

Industrial chemistry in the new millennium is widely adopting the concept of "Green Chemistry" to meet the fundamental scientific challenges of protecting the human health and environment while maintaining commercial viability. The emerging area of green chemistry envisages minimum hazard as the performance criteria while designing new chemical processes, one of the thrust areas for achieving the target is to explore alternative reaction condition and the media to accomplish the desired chemical transformation with minimized by products or waste as well as eliminates the use of conventional organic solvents. The microwaves couple directly with the molecules that are heating, leading to a rapid rise in temperature. The result is an instantaneous heating of anything that will react to dipole rotation or ionic conduction, the two fundamental mechanisms for transferring energy from microwaves to the substance being heated. The pharmaceutical importance of pyrazolines, which are of our interest, lies in the fact that they can be effectively utilized as antibacterial [1-5], antifungal [1, 3-5], anti-inflammatory [6], antitubercular [7], analgesic [8], insecticidal [9], antiparasitic [10] and antiviral [11] agents. Some of these compounds have also

anticonvulsant [12], cardiovascular [13] and anticancer [14] properties. In addition, pyrazolines have played a crucial part in the development of theory in heterocyclic chemistry and also used extensively in organic synthesis. The literature survey reveals the importance of pyrazolines as intermediates in dye industry and they are also useful as biodegradable agrochemicals. The present studies were performed with the objectives: microwave assisted synthesis of new series of pyrazoles, characterization by spectral methods and screening of the antimicrobial activity of the synthesized compounds.

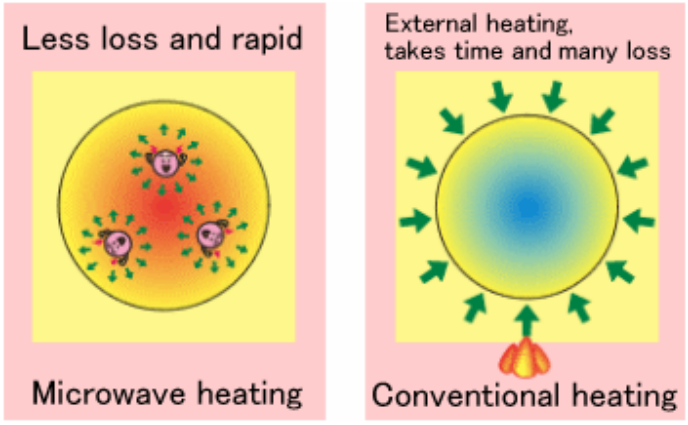


Fig.1. Microwave and conventional heating

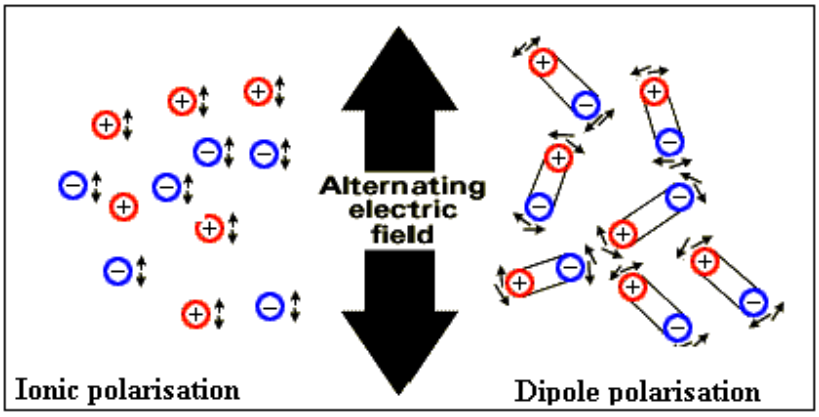
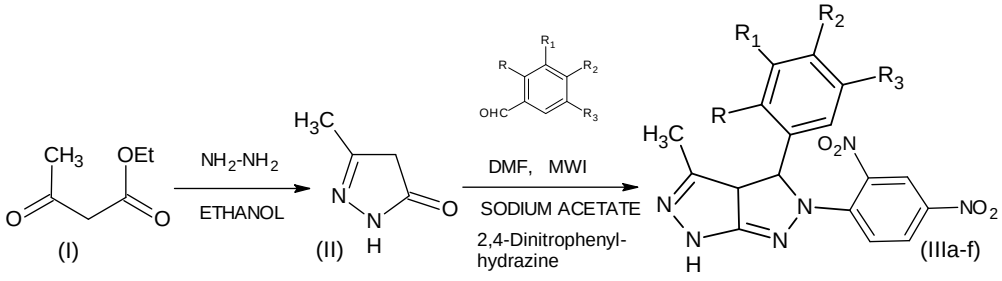


Fig.2. Molecular oscillations of polarisable substances under the influence of an alternating electric field



SCHEME

Compound	R	R_1	R_2	R_3
IIIa	H	H	OH	H
IIIb	H	NO_2	H	H
IIIc	H	H	Cl	H
IIId	Cl	H	Cl	H
IIIe	H	H	$N(CH_3)_2$	H
IIIf	H	OCH_3	OCH_3	OCH_3

MATERIALS AND METHODS

All reactions were carried out under prescribed laboratory conditions. All the reactions requiring anhydrous conditions were conducted in well dried apparatus. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary and their melting points were checked with the available literature. Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. The final products were purified by recrystalization and purity was checked by micro TLC. The IR spectra of the compounds were recorded on JASCO FT/IR-5300 spectrometer using KBr pellet. ^{1}H NMR spectra were recorded in a BRUKER DPX-400MHz spectrometer using TMS as internal standard. GCMS spectra were recorded in SHIMADZU QP 50000. Elemental analysis for C, H and N were performed on a PERKIN ELMER 240 C elemental analyzer and were found to be within ± 0.4 % of the theoretical values.

ANTIMICROBIAL ACTIVITY

Antibacterial activity: The newly synthesized compounds were evaluated for the antibacterial activity against bacterial strains by using zone of inhibition method [15]. The solutions of the test and standard drugs amoxicillin/clavulanic acid and cefixime were prepared at the concentration of 1000 µg/mL in DMSO. Previously sterilized, liquified Muller Hinton Agar media was inoculated with the requisite quantity of the suspension of the microorganism at a temperature between 40-50 ºC and the inoculated medium was poured immediately aseptically into Petri dish, previously sterilized to occupy a depth of 3-4 mm. The Whatmann filter paper no. 2 was cut down into a small disc (6 mm in diameter) sterilized in the hot air oven and then impregnated with the test solutions and standard solution. The dried discs were placed on the surface of the medium aseptically. All the Petri dishes were left standing for 1 to 4 h at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. All the Petri dishes were incubated for 24 h at 37 ºC. After incubation the diameters of the circular inhibition zones were measured.

Antifungal activity: The newly synthesized compounds were evaluated for the antifungal activity against fungal strains by using zone of inhibition method [15]. The solutions of the test drugs as well as the standard drug, ketoconozole were prepared at the concentration of 1000 µg/mL in DMSO seperately. After completion of a period of pre-incubation diffusion, all the Petri dishes were incubated for 48 h at 25 ºC. After incubation the diameters of the circular inhibition zones were measured.

Synthesis of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II) (conventional method)

Ethylacetoacetate (1.3g, 0.01mol) was placed in a conical flask and stirred magnetically during the slow dropwise addition of solution of hydrazine hydrate (98%,0.5 ml, 0.01 mol) in absolute ethanol (1ml) and temperature of about 60^0 C was maintained, a crystalline deposit was separated. After stirring for 1 h at room temp, the reaction mixture was cooled in an ice bath to complete recrystalisation, filtered, washed with ice-cold ethanol, dried, m.p.222^0 C. Yield 0.88g,90%.

General procedure

Synthesis of 5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IIIa-f)

A mixture of 5-methyl-2,4 dihydro-3*H*-pyrazol-3-one (II) (0.98g, 0.01mol), Aromatic aldehyde (0.01 mol), 2,4-dinitrophenylhydrazine (1.98g, 0.01 mol), was dissolved in DMF (40ml) in presence of anhydrous sodium acetate (0.82g, 0.01mol) and irradiated under microwaves (490 W) for 8 mins. Reaction completion was monitored by TLC (mobile phase: benzene), mixture was cooled, poured in ice water; crude product thus obtained was washed with cold ethanol. Yield and melting point were noted.

4-Phenyl-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c]pyrazole (IIIa): IR (KBr, cm^{-1}): 3287 (N-H), 1585 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.5 (d, 1H, C3a -H), 7.8 (d, 1H, C4-H), 7.2-7.5 (m, 8H, Ar-H), 6.6(s, 1H, N-H), 2.4 (s, 3H, -CH_3). M/e: 366; Anal. ($C_{17}H_{14}N_6O_4$) Found: C, 55.71; H, 3.84; N, 22.93 Calculated : C, 55.73; H, 3.85; N, 22.94%.

4-(2-Chlorophenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c] pyrazole (IIIb): IR (KBr, cm^{-1}): 3262 (N-H), 1520 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.7 (d, 1H, C3a-H), 6.9 (d, 1H, C4-H), 7.41-7.54 (m, 7H, Ar-H), 6.2 (s, 1H, N-H), 2.43 (s, 3H, CH_3). M/e: 400; Anal. ($C_{17}H_{13}ClN_6O_4$) Found: C, 50.55; H, 3.12; N, 21.65. Calculated : C, 50.95; H, 3.27; N, 20.97%.

4-(2-Hydroxyphenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c] pyrazole (IIIc): IR (KBr, cm^{-1}): 3428 (OH and N-H), 1509 (C=N). ^{1}H NMR ($CDCl_3$) δ: 9.1 (s, 1H, O<u>H</u>), 8.6 (d, 1H, C3a-<u>H</u>), 7.3-7.5 (m, 7H, Ar-<u>H</u>), 7.0 (d, 1H, C4-<u>H</u>), 6.3 (s, 1H, N-<u>H</u>), 2.5 (s, 3H, C<u>H</u>$_3$). M/e: 382; Anal. ($C_{17}H_{14}N_6O_5$) Found: C, 49.32; H, 3.68; N, 20.46 Calculated : C, 49.04; H, 3.87; N, 20.18%.

4-(2,4-Dichlorophenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c] pyrazole (IIId): IR (KBr, cm^{-1}): 3281 (N-H), 1581 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.0 (d, 1H, C3a-<u>H</u>), 7.2-7.5 (m, 6H, Ar-<u>H</u>), 6.8 (d, 1H, C4-<u>H</u>), 6.2 (s, 1H, N-<u>H</u>), 2.5 (s, 3H, C<u>H</u>$_3$). M/e: 435; Anal. ($C_{17}H_{12}Cl_2N_6O_4$) Found: C, 46.80; H, 2.54; N, 19.10 Calculated : C, 46.91; H, 2.78; N, 19.31%.

4-(4-Diaminomethylphenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c]pyrazole (IIIe): IR (KBr, cm^{-1}): 3281 (N-H), 1507 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.07 (d, 1H, C3a-<u>H</u>), 7.2 (d, 1H, C4-<u>H</u>), 7.37-7.45 (m, 7H, Ar-<u>H</u>), 6.5(s, 1H, N-<u>H</u>), 3.1 (s, 6H,-N (C<u>H</u>$_3$)$_2$), 2.5 (s, 3H, CH_3). M/e: 409; Anal. ($C_{19}H_{19}N_7O_4$) Found: C, 55.60; H, 4.32; N, 23.80 Calculated : C, 55.74; H, 4.68; N, 23.95%.

4-(3,4,5-Trimethoxyphenyl)-5-(2,4-dinitrophenyl)-3-methyl-1,3a,4,5-tetrahydropyrazolo-[3,4-c]pyrazole (IIIf): IR (KBr, cm^{-1}) 3268 (N-H), 1586 (C=N). ^{1}H NMR ($CDCl_3$) δ: 8.07 (d, 1H, C3a-<u>H</u>), 6.9 (d, 1H, C4-<u>H</u>), 7.39-7.56 (m, 5H, Ar-<u>H</u>), 6.2 (s, 1H, N-<u>H</u>), 3.1 (s, 9H, -(OC<u>H</u>$_3$)$_3$), 2.5 (s, 3H, C<u>H</u>$_3$). M/e: 456; Anal. ($C_{20}H_{20}N_6O_7$) Found: C, 52.46; H, 4.10; N, 18.24; Calculated : C, 52.63; H, 4.41; N, 18.41%.

RESULTS AND DISCUSSION

The physical data of synthesized compounds (IIIa-f) is given in **Table 1**, antibacterial activity is given in **Table 2** and antifungal activity is given in **Table3**

Table 1 Physical data of 4,5-disubstituted-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IIIa-f)

Compound	R	R_1	R_2	R_3	Reaction time (minutes)	Recrystalization solvent	%yield	m. p. (^{0}c)	Molecular formula	Molecular weight	Rf value
IIIa	H	H	H	H	8	Ethanol	68	226-227	$C_{17}H_{14}N_6O_4$	366.332	0.66
IIIb	Cl	H	H	H	8	Glacial acetic acid + Ethanol(1:1)	72	215-216	$C_{17}H_{13}ClN_6O_4$	400.777	0.44
IIIc	OH	H	H	H	8	Glacial acetic acid	69	206-208	$C_{17}H_{14}N_6O_5$	382.331	0.62
IIId	Cl	H	Cl	H	8	Glacial acetic acid	79	223-224	$C_{17}H_{12}Cl_2N_6O_4$	435.222	0.60
IIIe	H	H	$N(CH_3)_2$	H	8	Ethanol	74	244-246	$C_{19}H_{19}N_7O_4$	409.399	0.44
IIIf	H	OCH_3	OCH_3	OCH_3	8	Glacial acetic acid	80	234-236	$C_{20}H_{20}N_6O_7$	456.409	0.53

** R_f value was determined in benzene: acetone (1:1)*

ANTIMICROBIAL ACTIVITY

A drug is considered to have bacteriostatic or fungistatic activity when it inhibits the activity of bacteria or fungi respectively and bactericidal or fungicidal activity and when it kills bacteria or fungi. Important factors for antimicrobial activity are size of the inoculum, metabolic state of microbe, pH, temperature, duration of interaction, concentration of inhibitor and presence of interference substances. Antibacterial activity was carried out on four bacterial strains, namely *Streptococcus mutans* (gram positive), *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative), *Shigella dysenteriae* (gram negative) and antifungal activity was carried out on two fungal strains, namely *Candida albicans* and *Rhizopus oryzae.* The results are shown in Table 2 and 3. From the results, compound IIIa showed good activity against *Streptococcus mutans*. The zone of inhibition produced by this compound is more than that of standard used for *Streptococcus mutans*. It showed moderate activity against *Staphylococcus aureus*, *Shigella dysenteriae* and *Escherichia coli*. Compound IIIb showed good activity against *Streptococcus mutans* and it is moderately active against Shigella *dysenteriae*, *Escherichia coli.* Compound IIIc showed no significant antibacterial activity and showed less activity against fungus *Rhizopus oryzae*. Compound IIId showed moderate activity against *Escherichia coli*. Compound IIIe showed moderate activity against *Streptococcus*

mutans, *Shigella dysenteriae* and *Rhizopus oryzae a*nd good activity against *Candida albicans.* Compound IIIf showed good activity against *Staphylococcus aureus, Candida albicans* and *Rhizopus oryzae* and it is moderately active against *Shigella dysenteriae.*

Table 2 Antibacterial activity of 4,5-disubstituted-3-methyl-1,3a,4,5-tetrahydropyrazolo[3,4-c]pyrazoles (IIIa-f)

Microorganism	Diameter of zone of inhibition (mm)						
	Compound IIIa (1mg/ml)	Compound IIIb (1mg/ml)	Compound IIIc (1mg/ml)	Compound IIId (1mg/ml)	Compound IIIe (1mg/ml)	Compound IIIf (1mg/ml)	Standard* (1mg/ml)
Streptococcus mutans	15	**16**	NA	6	12	2	16
Staphylococcus aureus	8	NA	2	4	4	12	14
Shigella dysenteriae	16	12	NA	2	12	16	24
Escherichia coli	14	12	4	10	4	4	20

NA*: No activity at this amount of test compound or standard*
****Standard drugs****: Amoxicillin-clavulanic acid (for gram positive bacteria), Cefixime (for gram negative bacteria)*

Table 3 Antifungal activity of 4,5-disubstituted-3-methyl-1,3a,4,5-tetrahydropyrazolo [3,4-c]pyrazoles (IIIa-f)

Microorganism	Diameter of zone of inhibition (mm)						
	Compound IIIa (1mg/ml)	Compound IIIb (1mg/ml)	Compound IIIc (1mg/ml)	Compound IIId (1mg/ml)	Compound IIIe (1mg/ml)	Compound IIIf (1mg/ml)	Standard* (1mg/ml)
Candida albicans	8	12	8	6	22	24	26
Rhizopus oryzae	6	10	12	NA	16	18	22

NA*: No activity at this amount of test compound or standard*
****Standard drug****: Ketoconazole*

CONCLUSION

Microwave assisted synthesis of pyrazolopyrazoles is entirely possible. The yield is almost quantitative. The present method is mild, exceedingly efficient, very rapid and especially Eco-friendly. All the compounds were synthesized with good yield (68-80%). All the synthesized compounds exhibited antibacterial and antifungal activities. Compound IIIa and compound IIIb showed good activity against *Streptococcus mutans* and moderate activity against *Staphylococcus aureus, Shigella dysenteriae* and *Escherichia coli*. Compound IIIb showed moderate activity against fungus *Candida albicans* and *Rhizopus oryzae.* Compound IIIc showed no significant antibacterial activity and showed less activity against fungus *Rhizopus oryzae.* Compound IIId showed moderate activity against *Escherichia coli.* Compound IIIe showed moderate activity against *Streptococcus mutans, Shigella dysenteriae* and *Rhizopus oryzae* and good activity against *Candida albicans.* Compound IIIf showed good activity against *Staphylococcus aureus, Candida albicans* and *Rhizopus oryzae* and it is moderately active against *Shigella dysenteriae.*. The synthesized compounds apart from the antimicrobial activities, are believed to exert various other activities such as antiinflammatory, analgesic, anticonvulsant, CNS depressant, ulcerogenic, anthelmintic activities.

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