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# Polyethylene Glycol (PEG-400): As Green Reaction Media for Rapid Synthesis of Preparation of Isoxazolinederivatives and Its Antimicrobial Screening

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## ABSTRACT

We wish to describe an efficient and rapid synthesis of isoxazolines by the reaction of substituted pyrazol-5-one were condensed with hydroxylamine hydrochloride in presence of clay (pH =12.5) and PEG-400 as a green reaction media. Herein, we report the conventional condensation method of substituted pyrazol-5-one with hydroxylamine hydrochloride in PEG-400 as reaction solvent at mild reaction condition. Structures of the synthesized compounds were confirmed by the spectral analysis. Furthermore, all the synthesized compounds were evaluated for their antimicrobial screening against several pathogenic representatives, these newly synthesized compounds were screened for their antimicrobial activity against bacterial strain Escherichia coli (MTCC-443), Bacillus subtilis (MTCC-441), Staphylococcus aureus (MTCC-96) and Salmonella typhi. (MTCC-98). The antifungal activity was evaluated against Aspergillus niger (MTCC-1781), Aspergillus flavus (MTCC-3008), Candida albicans (MTCC-227) and Penicillium chrysogenum (MTCC-160).The result revealed that most of the compounds showed good to moderate Antimicrobial screening. The major advantages of this protocol are it gives excellent yields of products, work up procedure and isolation is easier, Procedure is green and environmentally benign, shorter reaction times.

**Keywords :** Pyrazolone, Isoxazoline, PEG-400, Clay(pH =12.5) andAntimicrobial activity.

## I. INTRODUCTION

Isoxazoline derivatives are useful as intermediates in the organic synthesis, polymers, pharmacologically active materials, dyes and pesticides.They are possessing fungicidal, antimicrobial, bactericidal and mutagenic activities.Isoxazolines possess various biological and pharmacological activities. In addition, they find application as dyestuffs, auxiliaries in fiber finishes, dropping dye in the electroluminescence device and in liquid crystalline mixture. Isoxazolines are biologically active, synthetically useful and important heterocycles having wide role in medicinal chemistry.Synthesis of novel isoxazoline derivatives remains a main focus of medicinal chemist, due to their diverse pharmacological activity. Isoxazoline derivatives have been reported to possessantibacterial[1],antifungal[2,3]anti-inflammatory[4], anticonvulsant[5], analgesic[6]andantiviral[7]activity.

Much research has been carried out with the aim to finding therapeutic values of isoxazolines moiety since their discovery. A large number of substituted isoxazoline derivativesare prepared and tested for variety of biological activities. Such as antimicrobial activity[8, 9]and hypolipemics[10].

Keeping these therapeutic interest and biological observations of isoxazolines in mind and in continuation of our research work on the synthesis of biologically active heterocyclic compounds [11-12], it was planned to synthesize some new series of isoxazolines derivatives.

## II. Experimental Section

Melting points were determined by an open capillary method and are uncorrected. The chemicals and solvents used are of laboratory grade and were purified. IR spectra were recorded (in KBr pallets) on Shimadzu spectrophotometer. 1H NMR spectra were recorded (in DMSO-d6) on Avance-300 MHz spectrometer using TMS as an internal standard. The mass were recorded on EI-shimadzu GC-MS spectrometer.

**General methods for the synthesis of Isoxazolines:**

A mixture of substituted pyrazol-5-one (0.001 mmol) and hydroxylamine hydrochloride (0.0015 mmol) was heated in (pH =12.5) and PEG-400 as a reaction media for 2-3 hrs. After completion of the reaction (checked by TLC), the contents were poured into ice-cold water. Solid get separated was filtered and washed by distilled water 20x2 mL, wet solid dried at 60-65°C, recrystallized from absolute ethanol to get pure isoxazolines . Aqueous MLR distilled under reduced pressure at 65-70°C to recover Poly (ethylene) glycol (PEG-400), which is reusable up to second recycle for same reaction. The structures of isoxazolines were confirmed by spectral analysis (IR, $^1$H NMR and MS).

**Spectral data of selected compounds:**

**1.3-(2-butyl-4-chloro-1H-imidazol-5-yl)-4-methyl-6-phenyl-3a,6-dihydro-3H-pyrazolo[3,4-] isoxazole(IIIa)**

IR (KBr): 3300, 3188, 2926, 1616, 1564, 820, 690 cm$^{-1}$.$^1$H NMR (DMSO-$d_6$) : δ 0.93 (t, 3H, -$CH_3$), 1.33 (m, 2H, -$CH_2$), 1.66 (m, 2H, -$CH_2$), 2.13 (s, 3H, -$CH_3$), 2.68 (t, 2H, -$CH_2$), 3.45-360 (dd, 1H, $H_a$), 4.4-4.6 (dd, 1H, $H_b$),7.2-7.9 (m, 5H, Ar- H), 8.3 (s, 1H, -NH, $D_2O$ exchangeable)ppm.Mass (m/z) [% rel. intensity]: 357[$M^+$ ion], 272[10], 254[45], 209[15], 183[18], 167[25],139[57], 111[30], 92[15], 77 [100], 65[40], 51[50].

**2.3-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4yl)-4-methyl-6-phenyl-3a,6-dihydro-3H-pyrazolo[3,4-c]isoxazole(IIIc).**

IR (KBr) : 3442, 3057, 2956, 1599, 1492, 748, 690 cm$^{-1}$.$^1$H NMR (DMSO-$d_6$) : δ 2.25 (t, 3H, -$CH_3$), 3.2-3.3 (dd, 1H, $H_a$) 4.45-4.6 (dd, 1H, $H_b$), 7.10-8.00 (m, 14H, Ar- H + s, (pyrazol ring)ppm.

Mass (m/z)[% rel. intensity]: 453[$M^+$ ion],407[8], 274[35], 231[10], 168[12], 155[100], 147[12], 119[20], 91[12], 76[12], 63[10].

**3.4-methyl-3-(3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-6-phenyl-3a,6-dihydro-3H-pyrazolo [3,4-c]isoxazole(IIId).**

IR (KBr: 3404, 3300, 2926, 1616, 1564, 839,690 cm$^{-1}$.$^1$H NMR (DMSO-$d_6$):δ 2.20 (t, 3H, -$CH_3$), 3.23-3.36 (dd, 1H, $H_a$), 4.50-4.66 (dd, 1H, $H_b$), 6.90-8.22 (m, 14H, Ar-H+ s, 1H, (pyrazol ring)ppm.Mass (m/z) [% rel. intensity]: 464[$M^+$ ion], 415[35], 359[34], 323[40], 282[26], 280[30],249[55], 232[10], 212[15], 185[18], 171[50], 92 [100],65[48], 43[40].

**Table 1.** physicoanalytical data ofSynthesized of Isoxazoline derivatives.

| Entry | Product No. | Mol. Formula | Yield % | M. P. (°C) |
|---|---|---|---|---|
| 1 | IIIa | $C_{18}H_{20}ClN_5O$ | 88 | 138-140 |
| 2 | IIIb | $C_{15}H_{14}N_4OS$ | 90 | 119-121 |
| 3 | IIIc | $C_{26}H_{20}ClN_5O$ | 86 | 154-156 |
| 4 | IIId | $C_{26}H_{20}N_6O_3$ | 88 | 156-158 |
| 5 | IIIe | $C_{17}H_{13}ClIN_3O_2$ | 92 | 120-122 |
| 6 | IIIf | $C_{18}H_{16}IN_3O_3$ | 90 | 113-115 |
| 7 | IIIg | $C_{17}H_{15}N_3O_2$ | 84 | 102-104 |
| 8 | IIIh | $C_{17}H_{14}ClN_3O$ | 86 | 92-94 |
| 9 | IIIi | $C_{17}H_{14}FN_3O$ | 88 | 90-92 |
| 10 | IIIj | $C_{19}H_{20}N_4O$ | 86 | 96-98 |

**General Mechanism:**

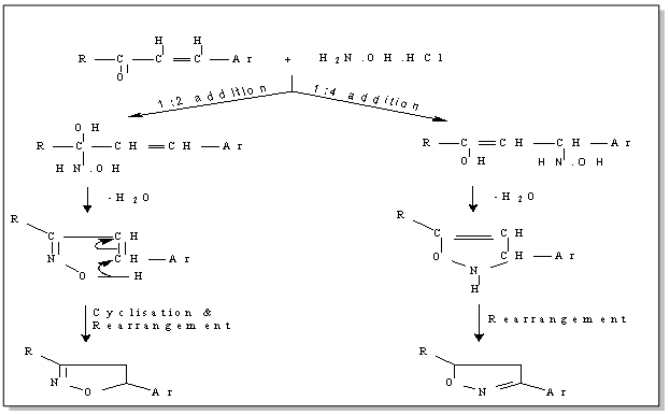
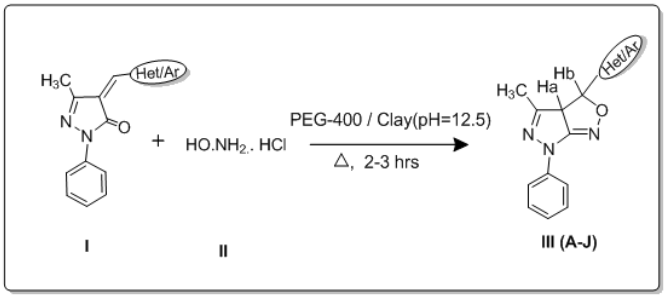


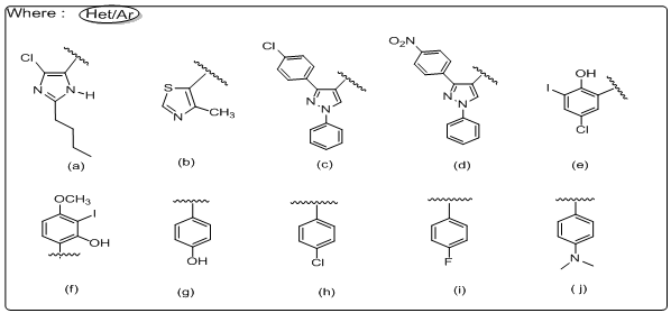

**Table 2.** Effect of solvent on the reaction of 3-(2-butyl-4-chloro-1H-imidazol-5-yl)-4-methyl-6-phenyl-3a,6-dihydro-3H-pyrazolo[3,4-c]isoxazole(IIIa)

| Entry | Solvent | Time (h) | Yield (%) |
|---|---|---|---|
| 1 | EtOH | 6 | 68 |
| 2 | THF | 6 | 75 |
| 3 | Dioxane | 4 | 74 |
| 4 | Acetonitrile | 5 | 67 |
| 5 | PEG-400 | 130(min) | 86 |

**Reaction Scheme:**




**Synthesis of isoxazolines from Pyrazol-5-ones with hydroxylamine hydrochloride:**




**Antimicrobial Screening:**

The antimicrobial activities of the synthesized compounds **III( a-j)**were determined by agarwell diffusion method[13].The compounds were evaluated for antibacterial activity against*Escherichia coli (MTCC-443)*, *Bacillus subtilis(MTCC-441), Staphylococcus aureus(MTCC-96)*and *Salmonella typhi. (MTCC-98)* The antifungal activity was evaluated against *Aspergillusniger*(MTCC-1781), *Aspergillus flavus (MTCC-3008), Candida albicans(MTCC-227)* and *Penicilliumchrysogenum(MTCC160)* were procured from Institute of Microbial technology (IMTech),Chandigarh, India. The antibiotic penicillin (25µg/mL) was used as reference drug forantibacterial and *Nystatin* (25µg/mL) used as antifungal activities. Dimethyl sulphoxide (1%, DMSO) was used a control without compound.

The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5 °C for 24hrs. Theantibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respectivebacterial culture strain suspension prepared in sterile saline (0.85%) of 105 CFU/mL dilutions.The wells of 6 mm diameter were filled with 0.1 mL of compound solution of concentration 25to 150µg/mL separately for each bacterial strain. All the plates were incubated at 37±0.5 °C for24 hrs. Zone of inhibition of compounds in mm were noted and minimum inhibitoryconcentrations (MICs) were noted. The results of antibacterial studies are given in Table 2. For antifungal activity, all the culture strains of fungi maintained on potato dextrose agar (PDA)slant at 27±0.2 °C for 24-48 hrs, till sporulation. Spore of strains were transferred in to 5 mL ofsterile distilled water containing 1% Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (106 CFU/mL). Sterile PDA plate was prepared containing 2% agar;0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27±0.2 °C for12 hrs. After incubation well prepared using sterile cork borer and each agar well was filled with0.1 mL of compound solution at concentration 25 - 150 µg/mL. The plates were kept inrefrigerator for 20 minutes for diffusion and then incubated at 27±0.2 °C for 24-28 hrs. Afterincubation, zone of inhibition of compounds were measured in mm along with standard andminimum inhibitory concentrations (MICs) were noted. The results of antifungal studies aregiven in Table 3.

**Table 3.** Antibacterial activity of synthesized compounds III(a-j)

| Product | *Escherichia coli(MTCC-443),* | *Bacillus subtilis(MTCC-441),* | *Staphylococcus aureus(MTCC-96)* | *Salmonella typhi. (MTCC-98)* |
|---|---|---|---|---|
| IIIa | 22(25) | 16(25) | 16(25) | 16(25) |
| IIIb | 18(25) | 15(25) | 18(25) | 14(25) |
| IIIc | 20(25) | 16(25) | 28(50) | 16(25) |
| IIId | 18(50) | 17(25) | 26(50) | 22(50) |
| IIIe | 22(50) | 11(50) | 11(50) | 30(50) |
| IIIf | 19(50) | 15(50) | 10(50) | 30(50) |
| IIIg | 29(50) | 12(50) | 25(50) | 18(50) |
| IIIh | 16(50) | 18(50) | 14(50) | ---- |
| IIIi | 20(50) | ------ | 16(50) | 32(50) |
| IIIj | 26(50) | 14(50) | ----- | 18(50) |
| Reference-1 | 24(25) | 18(25) | 24(25) | 18(25) |

**Table 4.** Antifungal activity of synthesized compounds III( a-j)

| Product | *Aspergillus niger*(MTCC-1781) | *Aspergillus flavus (MTCC-3008)* | *Candida albicans (MTCC-227)* | *Penicillium chrysogenum(MTCC160)* |
|---|---|---|---|---|
| IIIa | 20(25) | 17(25) | 16(25) | 16(25) |
| IIIb | 19(25) | 20(25) | 17(25) | 15(25) |
| IIIc | 19(25) | 22(25) | 14(25) | 14(25) |
| IIId | 15(25) | 14(25) | ----- | 17(25) |
| IIIe | 18(50) | 10(50) | 20(50) | 19(50) |
| IIIf | 19(25) | 19(25) | 18(25) | 14(25) |
| IIIg | 14(50) | ----- | 15(50) | ----- |
| IIIh | 18(50) | 14(50) | 12(50) | 16(50) |
| IIIi | 14(50) | 15(50) | ------ | 15(50) |
| IIIj | 15(50) | 12(50) | 18(50) | 15(50) |
| Reference-2 | 20(25) | 18(25) | 22(25) | 18(25) |

Zone of inhibitions are expressed in mm, MIC values (mg/mL) are given in brackets. Reference-1=Penicilllin, Reference-2=Nystatin, -- MIC > 50 mg L –1, Solvents: DMSO, water

The examination of the data Table (3) and Table (4) reveals that majority of the compounds showed antibacterial and antifungal activity when compared with standard drug. The results of in vitro antibacterial activities of compounds III( a-j)against various bacterial strains are summarized in Table 3. It has been observed that some of compounds exhibited interesting antibacterial activity. In comparison with reference antibacterial, compounds IIIa, IIIb and IIIc shows good zone of inhibition against *Escherichia coli* as well as Compounds IIIa,IIIc and IIId were also displayed comparative activity against *Bacillus subtilis*. Compounds IIIa and IIIb display moderate to good activity against *Staphylococcus aureus*. IIIa and IIIc shows promising activity against *Salmonella typhi.*IIIh, IIIi, and IIIj display reduced activity against all tested bacteria.

Antifungal data in Table 4 revealed that compounds IIIa, IIIb, IIIc andIIIf showed good to moderate activity against *Aspergillus niger*. Compounds IIIa, IIIb, IIIf and IIIc were showed most promising activity compared to standard antifungal against *Aspergillus flavus*. Compounds IIIb and IIIf were showed good activity against *Candida albicans*. Only the compound IIIa,IIIb and IIId was showed stronger activity compared with standard drug against Penicillium chrysogenum. When structure activity relationships are concerned, the antimicrobial activity might be increased by the presence of halo (I, Br and Cl) groups as substituents position.

Considering the results obtained from antibacterial and antifungal activities, it is possible to say that most of the tested compounds showed good zone of inhibition against bacteria and fungi also the minimum inhibitory concentrations (MICs). Therefore, the present study is useful drugs in medicinal investigation against bacterial and fungal diseases.

## III. Result and Discussion

In continuation of our work on the synthesis of some new bioactive heterocyclic compounds [14-15],herein we report new series of Isoxazoline derivatives by the condensation of pyrazol-5-onewith hydroxylamine hydrochloride using basic clay in polyethylene glycol (PEG-400) as a green reaction solvent**.**The reaction went to completion within 130 to150 minutes and corresponding product III**(a-j)** was obtained in 86-92% yield. In order to optimize the reaction conditions, we carried out the above reaction in different solvents such as ethanol, tetrahydrofuran, dioxane, acetonitrile and polyethylene glycol-400 (Table2). We found that polyethylene glycol-400 as an efficient reaction medium in terms of reaction time as well as yields (86-92%). Encouraged by the results, we turned our attention to variety of substituted isoxazoline derivatives. In all cases, the reaction proceeded efficiently in high yields at 60°C using PEG-400 as an alternative reaction solvent. Again these synthesized compounds were characterized by IR,NMR and Mass.The IR spectra showed characteristic absorption band at 1590-1620 $cm^{-1}$ due to C=N stretching. Beside these bands 680–800 $cm^{-1}$ due to C-Cl stretching,$^{1}$H NMR of the isoxazoline showed following type of peak which confirmed the formation of product and mass spectra confirmed representative molecular weight of the compound. These newly synthesized compounds were screened for their Antimicrobial screening, the results obtained from antibacterial and antifungal activities, it is possible to say that most of the tested compounds showed good zone of inhibition against bacteria and fungi also the minimum inhibitory concentrations (MICs).

## IV. Conclusion

In conclusion, our protocol is a practical approach which uses of Clay(pH =12.5)) and PEG-400as a commercially

available, low-cost, easily available solvent. In most cases, the reaction proceededsmoothly to produce the correspondingderivatives. The reactionwas clean and the products were obtained in excellent yields without formation of anyside products.

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