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# Conventional & Microwave-Assisted Synthesis and Antimicrobial Evaluation of Pyrimidine Azo Compounds

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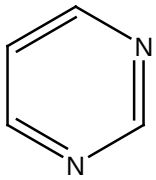
**Abstract:** ***Pyrimidine derivatives are an important class of heterocycles and significant bioactive moieties with wide rangeof biological activities such as, antibacterial, anti- inflammatory, antihypertensive, antitumor and calcium channel blocking activity. The coupling of certain pyrimidine derivatives (e.g. barbituric acid) with azo compounds and their further metallation with transition metals give rise to bioactive moieties along with diverse industrial applications. This study will report the synthesis and characterization of pyrimidine complexes and its derivatives by conventional and microwave-assisted synthetic routes. Furthermore, both methods will be compared to elaborate the advantages associated with microwave-assisted synthesis.***

**Keywords:** azo compounds, barbituric acid, pyrimidine complexes, microwave assisted method

## 1. Introduction

Pyrimidines are organic aromatic heterocyclic compounds.The basic skeleton of a pyrimidine ring is composed of two elements i.ecarbon and nitrogen, and are also named as diazines having two nitrogen atoms at position 1 and 3 [1].




pyrimidine

The significant position of pyrimidine and its derivatives in Organic Chemistry is primarily related to their bioactivity. Above of all, they constitute nucleic acids which is the base of life. Three nucleobases (cytosine, thymine and uracil) are pyrimidine derivatives [1].

The biodynamic property of pyrimidine ring structure has urged the medicinal chemists to synthesize such pyrimidine derivatives which can stimulate pharmacophores and can be utilized for various pharmacological applications. The core structure of pyrimidinehelps them by offering certain reaction sites that can be used to react furtherwith different moieties. Azo compounds are one of those compounds that are known widely to react with pyrimidine rings.Interestingly, they also demonstrate bioactivities that make them important species in medicinal chemistry. The azo moieties are used as chemotherapeutic agents in detecting cancer and as hypnotic drugs [2]. Moreover, Pyrimidine azo coupling reactions are well known. Compounds synthesized as a result of these reactions are colored so they can be applied in many industrial applications such as fiber coloring, printing system, photo electronic, biological reactions, in field of analytical chemistry, technology of optical storage[3] and finding trace metals in water and food items [4]. Above of all,bioactivity of Pyrimidine azo compounds is enormous including anti-inflammatory, antibacterial, antifungal activities that are reported in literature [4]. Furthermore transition metal complexes of the pyrimidine azo compounds can also be synthesized with therapeutic potencies.

The present research work was designed to synthesize pyrimidine azo compounds and their metal complexes by adopting conventional synthesis reported in literature and then its comparison with a more convenient and ecofriendly technique that is “microwave-assisted synthesis”.

Microwave-assisted synthesis is acknowledged as a major breakthrough in synthetic chemistry in recent years. This technique has overcome the certain backdraws associated with conventional routes i.e. larger reaction time, reduced yields and purity and slow rate of reaction. Microwave synthesis provides more opportunities to organic chemists to expand their synthetic avenues by applying microwave irradiation to a variety of organic reactionswith improved results [5].

## 2. Literature Survey

Sixteen antimicrobial metal complexes of uric acid with different interactions and stereochemistries were prepared (Na, K, UO2, Cd, Cu, Ni, Co, Fe, Mn and Cr) [6] and another antimicrobial 2-arylhydrazono-3

ketiminobutyronitriles has been synthesized by diazotization of aniline derivatives and coupling reaction with 3-aminocrotononitrile [7]. Pyrimidine azo dyes 5-(quinoly-8-azo)-1, 3-dimethylpyrimidine-2, 4, 6-trione ($L_2$) and 5-(quinoly-8-azo) pyrimidine-2, 4, 6-trione were obtained by diazo coupling reaction of barbituric acid and 8-aminoquinoline.reaction of these azo dyes with metal salts like Ni or Cu salts provided mononuclear metal complexes [8]. A heterocycleazo dye was obtained with diazotization of 5-Amino-4-arylazo-3-methyl-1H-pyrazoles and coupling with barbituric acid [9]. Antifungal metal complexes of Pt, Pd, Ni, Co and Mn with macro cyclic ligands 2, 3, 9, 10-tetraketo-1, 4, 8, 11-tetraazacycoletradecane [10] are also reported in literature.

## 3. Materials and Method

All chemicals used in the synthesis were of analytical grade from Merck and Fluka. Microwave oven DW-180, 2450 MHZ, 950W was used for synthetic purpose.

Widespread scope of bioactivities of pyrimidine azo compounds along with their metal complexes is the tempting point for chemists to adopt simple and efficient routes for synthesizing these bioactive moieties. These compounds were synthesized by both reported conventional methods as well as using microwave technology. The results produced by both strategies were compared for the evaluation of advantages claimed by microwave-assisted synthesis. Anti-bacterial and anti-fungal properties associated with synthesized compounds were determined.

The synthesis of compounds was accomplished in two steps.
1. Synthesis of pyrimidine azo compound that acts as ligand (L)
2. Metalltion of ligand with transition metals (M) i.e. Cu, Ni and Co.

### 3.1. Synthesis of ligands

#### 3.1.1 Synthesis of $L_1$
9.3g of aniline was mixed with hot water and 10mL conc. HCl and it was diazotized with $NaNO_2$ solution (0.7g in 8.0mL water). Diazotized salt was reacted with barbituric acid solution (1.54g of barbituric acid in presence of sodium acetate and 20 mL ethanol. Yellow crystals were obtained after filtration followed by washing with methanol and recrystallization withdimethylformamide and charcoal.

#### 3.1.2 Synthesis of $L_2$
Solution of 1.371g anthranilic acid in HCl and water was diazotized with $NaNO_2$ solution (6.89 g per 20 mL). Then it was coupled with alkaline solution of barbituricacid . The product was crystallized with acetic acid and then recrystallized with water.

#### 3.1.3 Synthesis of $L_3$
Solution of 1.188g of 2-aminopyrimidine was prepared in 2.0 mL $H_2SO_4$ 9.0 mL water. Diazotization was accomplished with $NaNO_2$ solution (0.862g in 2.0 mL water). The diazotized compound was reacted with barbituric acid solution in water in presence of $Na_2CO_3$ solution (2.65g in 15mL water). The pH was maintained at 6 after stirring for 6 hours. Mixture was left overnight, filtered, washed with ethanol and recrystallized with water.

### 3.2 Metallation of ligands with transition metals:

Metalltion of synthesized ligands was done with conventional route and then with microwave irradiation. Three metals Cu, Ni and Co were reacted with each ligand.

#### 3.2.1 Conventional Synthesis
**(a) Conventional synthesis of $ML_1$**
Solution of 1.0g of $L_1$ in 30mL ethanol was mixed with metal salt solutions (1.89g of Cu (II), Ni (II) and Co(II) in 10mL ethanol) turn by turn. Mixture was refluxed in oil bath for 2 hours at 78$^o$C. Product was obtained after cooling precipitated. Then it was filtered and washed with ethanol.

**(b) Conventional synthesis of $ML_2$**
Ligand solution (1.0g of ligand $L_2$ in water) was reacted with ammoniacal solution of metal salt (1.349 g of metal salts i.e. Cu (II), Ni (II) and Co(II)) followed by reflux for 2 hours. Precipitates were kept overnight, filtered and dried after washing with ethanol.

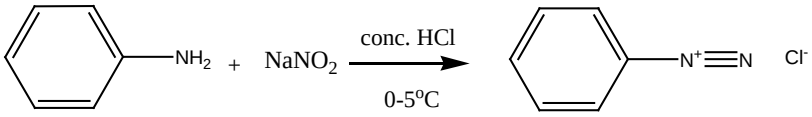
**(c) Conventional synthesis of $ML_3$**
0.5g of ligand $L_3$ in 15 mL ethanol was mixed with metal salt solution (0.95g of Cu (II), Ni(II), Co(II) metal salten inn 5.0 mL ethanol in presence of ammonia 2.0mL. Then reflux in oil bath for 2 hours was carried out. The precipitates were obtained after filtration and washing with ethanol.

#### 3.2.2 Microwave-assisted synthesis:
**(a) Microwave- assisted synthesis of $ML_1$**
Mixture of 0.25g ligand $L_1$ solution in 10mL ethanol was mixed with metal salt solutions (0.63g of each salt in 3.33mL of ethanol) and ammonia solution. Then reaction mixture was irradiated under microwave radiations for 30 seconds. Resulting product was obtained after washing with ethanol.

**(b) Microwave- assisted synthesis of $ML_2$**
Ligand $L_2$ (0.25g) was dissolved in water and after mixing ammoniacal solution of metal salts (0.45g), mixture was reacted under microwave radiations for 36 seconds. Precipitates were filtered, washed with ethanol and then dried.

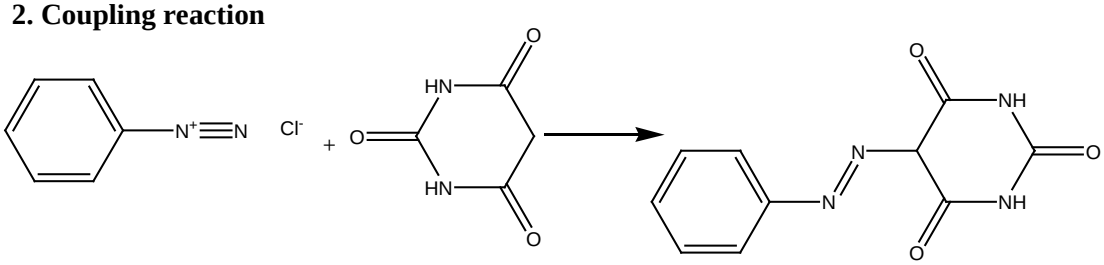
**(c) Microwave- assisted synthesis of $ML_3$**
0.25 g of ligand in 7.0mL ethanol was irradiated in microwave oven for 40 seconds after mixing 0.45g of metal salt solution of each metal in 2.5mL ethanol. After filtration it was washed with ethanol and then dried.

**1. Diazotization reaction**



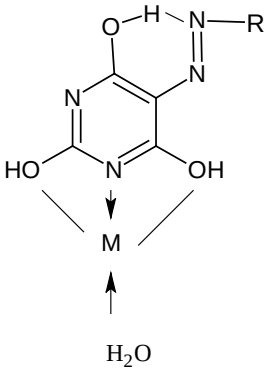

Benzene Diazonium chloride

**2. Coupling reaction**




Barbituric acid 5-[phenylazo]barbituric acid

**3. Metal complexation**




$H_2O$

R=Aniline, Anthranilic acid, 2-aminopyrimidine

M= Co (II), Ni (II), Cu (II)

**Figure 1:** Schematic diagram of synthesis of pyrimidine derivatives

## 4. Results& Discussion

The percentage yield of synthesized compounds and time taken by the reactions was recorded (Table 1and Table 2).

**Table 1:** Percentage yield of synthesized compounds

| *Compound* | *Percentage yield (%)* | |
|---|---|---|
| | Conventional | Microwave |
| $L_1$ Cu | 35% | 47% |
| $L_1$ Ni | 65% | 74% |
| $L_1$ Co | 57% | 75% |
| $L_2$ Cu | 31% | 45% |
| $L_2$ Ni | 26% | 39% |
| $L_2$ Co | 40% | 55% |
| $L_3$ Cu | 26% | 33% |
| $L_3$ Ni | 55% | 69% |
| $L_3$ Co | 44% | 52% |

**Table 2:** Reaction time of compounds

| *Compound* | *Time(s)* | |
|---|---|---|
| | Conventional | Microwave |
| $L_1$Cu | 7200 | 40 |
| $L_1$Ni | 7200 | 36 |
| $L_1$ Co | 7200 | 38 |
| $L_2$ Cu | 7200 | 30 |
| $L_2$Ni | 7200 | 47 |
| $L_2$Co | 7200 | 30 |
| $L_3$Cu | 7200 | 39 |
| $L_3$Ni | 7200 | 40 |
| $L_3$ Co | 7200 | 40 |

Melting points could not be recorded because these compounds decompose upon heating upon reaching a certain limit.

UV spectra were recorded within the range 200-600nm on Hitachi U-2800 spectrophotometer.

**Table 3:** UV/VIS data of synthesized compounds

| *Compound* | *Conventional* $\lambda_{max}$ *(nm)* | *Microwave* $\lambda_{max}$ *(nm)* |
|---|---|---|
| $L_1$ Cu | 422 | 421 |
| $L_1$ Ni | 397 | 396 |
| $L_1$ Co | 395 | 398 |
| $L_2$ Cu | 529 | 530 |
| $L_2$ Ni | 470 | 469 |
| $L_2$ Co | 470 | 473 |
| $L_3$ Cu | 583 | 580 |
| $L_3$ Ni | 569 | 570 |
| $L_3$ Co | 521 | 520 |

UV-VIS data for metal complexes synthesized by both conventional and microwave irradiation is in close approximation with each other. Low values of $\lambda_{max}$ are due to $\pi - \pi^*$ and higher values are due to n-$\pi^*$ transitions (table-3). The UV/Vis data of ligands showed absorption bands within the range of 290nm and 285nm respectively. On the other hand the complexes show decreased absorbance as a result of chelation.

FTIR spectra of all synthesized compounds were recordedonMidac USA M-2000 FTIR Spectrophotometer. (Table 4a, 4b). FTIR spectra for all synthesized compounds showed approximately similar values for conventional and microwave-assisted method. The IR bands at region of 3400 – 3500 $cm^{-1}$ are due to presence of intermolecular hydrogen bond, strong bands at 16650 – 1750 $cm^{-1}$ region are assigned to C==O, 1445–1500 $cm^{-1}$ are due to C==N bonding and band at 400-550 show M–O bonding in metal complexes.The FTIR spectral data shows a band of metal-oxygen and metal-nitrogen bond in all the metal complexes at the range of 400-500 $cm^{-1}$ which was not present in the ligands indicating the formation of metal complexes.

Mass spectra of all metal complexes synthesized by microwave irradiation method were taken by **GCMS Schimadzo QP-2010 Spectrometer**. Mass spectra of all metal complexes exhibited their base peaks. Peaks at 169 and 43 are evident due to fragmentations of N==N and M–O bond.

**Table 4 (a):** FTIR data of compounds synthesized by conventional method

| *Compound* | *Conventional*<br>*Wave number ($cm^{-1}$) Absorption Intensity* |
|---|---|
| $L_1$ Cu | 476.04, 540.22, 601.37, 772.51, 839.15, 1137.94, 1201.22, 1268.44, 1306.46, 1383.72, 1463.73, 1516.17, 1629.45, 3219.66, 3411.82, 3471.30 |
| $L_1$ Ni | 490.17, 599.95, 1121.83, 1204.21, 1396.36, 14499.91, 1544.78, 1625.50, 2347.58, 2921.64, 3262.34, 3420.798 |
| $L_1$ Co | 470.50, 607.66, 768.65, 1116.61, 1392.84, 1549.588, 1617.85, 1715.50, 2356.0531, 3211..953, 3449.89 |
| $L_2$ Cu | 610.650, 679.724, 770.167, 1384.673, 1508.868, 1636.334, 2343.231, 2624.747, 3409.876, 3607.996, 3798.891, 3838.536, 3957.735 |
| $L_2$ Ni | 706.31, 759.48, 1396.77, 1625.65, 1710.63, 2343.23, 3304.44, 3491.946, 3589.176, 3740.94, 3782.11, 3830.70, 3876.049, 3971.572 |
| $L_2$ Co | 549.74, 833.77, 1200.51, 1383.68, 1516.29, 1620.43, 3404.09, 3448.85, 3737.59, 3782.74, 3822.69, 3886.95, 3950.94 |
| $L_3$ Cu | 426.92, 496.25, 837.45, 1376.86, 1499.97, 1641.63, 1738.48, 3850.45, 3909.42 |
| $L_3$ Ni | 408.02, 442.76, 515.59, 829.98, 1163.30, 1261.20, 1433.01, 1512.84, 1655.34, 1746.04, 3252.66, 3784.18 |
| $L_3$ Co | 745.99, 833.10, 1162.75, 1261.68, 1514.09, 1653.78, 1742.45, 3483.23, 3559.47, 3882.86 |

**Table 4 (b):** FTIR data of compounds synthesized by MW

| *Compound* | *Microwave*<br>*Wave number ($cm^{-1}$) Absorption Intensity* |
|---|---|
| $L_1$Cu | 471.04, 516.83, 773.17, 1239.85, 1378.55, 1428.81, 1592.41, 1664.64, 1724.01, 260319, 3271.97, 3442.92 |
| $L_1$Ni | 510.85, 605.25, 1129.90, 1273.18, 1388.177, 1505.18, 1617.39, 2353.996, 2721.74, 3182.668, 3403.36 |
| $L_1$Co | 453.096, 599.455, 1122.98, 1199.51, 1389.09, 1600.94, 2359.017, 3432.98 |
| $L_2$Cu | 610.65, 679.72, 770.17, 1384.67, 1508.9, 1636.33, 2343.23, 2624.75, 3409.9, 3607.99, 3798.9, 3838.54, 3957.73 |
| $L_2$Ni | 690.41, 764.70, 1390.98, 1614.96, 1716.09,2963.29, 3554.397, 3585.07, 3675.16, 3721.788, 3775.94, 3847.18, 3901.668, 3967.639 |
| $L_2$Co | 545.27, 698.976, 759.18, 840.16, 906.899, 969.13, 1027.83, 1069.43, 1369.994, 1447.86, 1493.01, 1600.68, 1805.70, 1873.19, 1945.88, 2343.23, 2851.21, 2927.42, 3026.61, 3061.01, 3743.87 |
| $L_3$Cu | 426.92, 496.25, 837.45, 1376.86, 1499.97, 1641.63, 1738.48, 3850.45, 3909.42 |
| $L_3$Ni | 408.02, 442.76, 515.59, 829.98, 1163.30, 1261.20, 1433.01, 1512.84, 1655.34, 1746.04, 3252.66, 3784.18 |
| $L_3$Co | 745.99, 833.10, 1162.75, 1261.68, 1514.09, 1653.78, 1742.45, 3483.23, 3559.47, 3882.86 |

The antibacterial evaluation of compounds synthesized was studied with the stains of *Bacillus subtilis*, both the ligands and their metal complexes showed antibacterial activity.

The antifungal activity was evaluated with the culture of *Aspergillus Niger*. All the compounds showed antifungal activity at concentrations of 50µg/ml and 25µg/ml.

Inhibition zone of bacterial growth were measured in cm. All metal complexes synthesized by microwave irradiation method showed good activity against *Bacillus subtilis* bacterium. The activity results showed that activity of these compounds was due to coordination of mixed ligands to metal ions. Nickel complexes showed maximum activity with all ligands ($L_1$, $L_2$ and $L_3$) as compared to other metal complexes (Table-5).

All ligands and their metal complexes (Cu, Ni, Co) solutions of different concentrations were tested against fungus *Aspergillus Niger*.. All compounds gave positive results in higher concentrations (50 mg/mL, 25 mg/mL and 12.5 mg/mL).(Table 6a, 6b).

**Table 5:** Inhibition zone showed by bacterial species

| *Compound* | *Inhhibition zone (cm)* |
|---|---|
| $L_1Cu$ | 2.2 |
| $L_1Ni$ | 2.8 |
| $L_1Co$ | 3.0 |
| $L_2Cu$ | 2.5 |
| $L_2Ni$ | 3.4 |
| $L_2Co$ | 2.5 |
| $L_3Cu$ | 2.3 |
| $L_3Ni$ | 3.1 |
| $L_3Co$ | 2.9 |

**Table 6(a):** Antifungal activity of compounds

| *Compound* | *Synthesized dilutions* | | |
|---|---|---|---|
| | *50mg per ml* | *25mg Per ml* | *12.5mg Per ml* |
| $L_1$ | + | + | + |
| $L_2$ | + | + | + |
| $L_3$ | + | + | + |
| $L_1$ Cu | + | + | - |
| $L_1$ Ni | + | + | + |
| $L_1$ Co | + | + | - |
| $L_2$ Cu | + | + | - |
| $L_2$ Ni | + | + | + |
| $L_2$ Co | + | + | + |
| $L_3$ Cu | + | + | + |
| $L_3$ Ni | + | + | + |
| $L_3$ Co | + | + | + |

**Table 6(b):** Antifungal activity of compounds

| *Compound* | *Synthesized dilutions* | | |
|---|---|---|---|
| | *6.25mg Per ml* | *3.125mg Per ml* | *1.575mg Per ml* |
| $L_1$ | - | - | - |
| $L_2$ | - | - | - |
| $L_3$ | - | - | - |
| $L_1Cu$ | - | - | - |
| $L_1Ni$ | - | - | - |
| $L_1Co$ | - | - | - |
| $L_2Cu$ | - | - | - |
| $L_2Ni$ | - | - | - |
| $L_2Co$ | - | - | - |
| $L_3Cu$ | - | - | - |
| $L_3Ni$ | - | - | - |
| $L_3Co$ | _ | _ | - |

## 5. List of abbreviations

| *Sr. No.* | *Compound Name* | *Abbreviations* |
|---|---|---|
| 1. | 5-[phenylazo] pyrimidine 2, 4, 6-trione | $L_1$ |
| 2. | 5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione | $L_2$ |
| 3. | 5-(pyrimidinil-2-azo) barbituric acid | $L_3$ |
| 4. | Copper complex of 5-[phenyl azo] pyrimidine 2, 4, 6-trione | $CuL_1$ |
| 5. | Nickel complex of 5-[phenyl azo] pyrimidine 2, 4, 6-trione | Ni $L_1$ |
| 6. | Cobalt complex of 5-[phenyl azo] pyrimidine 2, 4, 6-trione | Co $L_1$ |
| 7. | Copper complex of 5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione | Cu $L_2$ |
| 8. | Nickel complex of 5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione | Ni $L_2$ |
| 9. | Cobalt complex of 5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione | Co $L_2$ |
| 10. | Cu-complex of 5-[2-pyrimidinylazo) pyrimidine 2, 4, 6 trione | Cu $L_3$ |
| 11. | Ni-complex of 5-[2-pyrimidinylazo) pyrimidine 2, 4, 6 trione | Ni $L_3$ |
| 12 | Co-complex of 5-[2-pyrimidinylazo) pyrimidine 2, 4, 6 trione | Co $L_3$ |

## 6. Conclusion

This reported research work was designed to synthesize bioactive pyrimidine azo compound and their metal complexes by using simple and eco-friendly synthetic methods and their comparison with that of conventional ones. Reduced reaction time, increased reaction rate and improved yield with high purity makes this protocol as competent and trouble-free synthetic strategy.

The structural elucidation of synthesized compounds were carried out via FTIR, UV/Vis, GC-MS and the antimicrobial (antibacterial and antifungal) activities of compounds were also studied. Although, compounds synthesized by both methods were in close agreement in the terms of their outputs. But microwave-assisted technique has demonstrated enormous advantages over the conventional ones and can be opted as first choice by synthetic chemists.

## 7. Future Scope

Microwave-assisted synthesis has been proved an efficient synthetic route to synthesize a large number of organic compounds. Its advantages over conventional ones are a breakthrough in organic synthesis. By using this eco-friendly methodology, better results can be produced in reduced time and cost. It can be used as key source to synthesize a large number of bioactive compounds on large scale.

## 8. Acknowledgement

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## Author Profile

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