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Synthesis and Characterization of Pyridine and Pyrazole Derivatives for Their Biological Activities

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Abstract : *Natural cyclic compounds have attracted more attention for several pharmacological applications. Pyridine and pyrazole activities can be augmented through the formation of heterocyclic compounds. In this study, we synthesized and evaluated novel heterocyclic compounds containing pyridine and pyrazole. The physiochemical characteristics revealed the structural properties of the synthesized compounds. The compounds were evaluated for their antibacterial and antifungal activities, which showed significant antimicrobial properties. The antioxidant activities analyzed by DPPH free radical scavenging activity that showed concentration dependent scavenging activities of the synthesized pyridine and pyrazole compounds. These results revealed that the synthesized compounds could be further evaluated for preparing lead antimicrobial and antioxidant pharmaceutical agent.*

Key words: *Pyridine, Pyrazole, Antimicrobial, Antioxidant*

I. Introduction

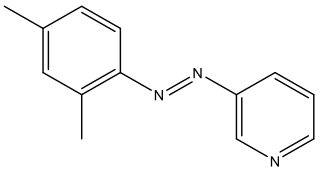
The indiscriminate use of antibiotics leads to development of resistant pathogens, which cause clinical problems including hypersensitivity, immune suppression and allergic reactions (Karaman et al., 2003; Mukherjee, Saritha, & Suresh, 2002). The free radicles formation induced by the infection as wells as disease conditions such as diabetic and cancer gives oxidative stress to the cells. Scavenging these elevated free radicles become effective therapeutic approach for efficient treatment(Farbstein, Kozak-Blickstein, & Levy, 2010). The developing countries lack the access of advanced drugs due to the economic constrains and the search for novel compounds, which possess multifunctional activities are still continues to treat infectious diseases (Puerstinger, Paeshuyse, De Clercq, & Neyts, 2007). Various heterocyclic derivatives containing nitrogen atom serve as a backbone for versatile scaffolds in designing drugs and biomolecules (Horton, Bourne, & Smythe, 2003). Among, the heterocyclic compounds containing pyridine and pyrazolerings are found to have diverse pharmacological properties including antimicrobial, antioxidant and anticancer activities. These compounds are frequently found as a part of biomolecules such as enzyme, vitamins in our body and also in natural products(El-Gohary & Shaaban, 2015; El-Sayed, Metwally, Nada, Mohamed, & Abdel-Rahman, 2013; Elassar, 2004; Fadda, Rabie, Etman, & Fouda, 2015; Gad-Elkareem, Abdel-Fattah, & Elneairy, 2011; Ghoneim, El-Farargy, & Abdelaziz, 2014; Klimesova, Svoboda, Waisser, Pour, & Kaustova, 1999; Mungra, Patel, & Patel, 2009; Patel, Agravat, & Shaikh, 2011).Pyrazole is a five-membered heterocyclic ring containing two adjacent nitrogen and the pyridine is a six-membered heterocyclic ring contains only one nitrogen, both known to have many applications. On the other hand, Anilines are important building blocks for important molecules such as indoles, which featured in various therapeutic agents. For example, the widely used antifungal agent itraconazole and antibacterial ciprofloxacin contain piperazineunit with aryl-group substitution(Cabeza et al., 2016; Karrouchi et al., 2018; Khan et al., 2016; Martins et al., 2013; Shetty & Bhagat, 2008). In this study, we synthesized pyridine and pyrazole derived azo compounds, characterized and analyzed for their biological activities.

II. Materials and methods

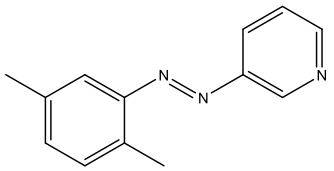
A. Synthesis of pyridine azo compounds

The pyridine derivatives were synthesized by diazotization of amines followed by coupling with pyridine as described earlier. (ref) In brief, solutions of aromatic amines (10 mmol) were prepared by dissolving 2,4-Dimethylaniline (29mg), 2,5-Dimethylaniline (29mg), 3,5-Dimethylaniline (29mg), Ortho Nitroaniline (33mg) Meta Nitroaniline (33mg) and Para Toluidine (25.7mg) in 6 ml of 3M HCl and 8 ml of water individually. The mixtures were cooled to 0°C in an ice bath with stirring. Once this solution was cooled to 0-5°C, 10 ml of freshly prepared 1M sodium nitrite solution was added slowly, maintaining the temperature below 5°C. The solution

was kept in an ice bath. 10 mmol pyridine solution was prepared by dissolving 47.5 mg of pyridine in 60 ml of 2M sodium hydroxide, and cooled to 0-5°C in an ice bath. 10ml of this solution was slowly added to the cooled substituted diazonium chloride solutions of aromatic amines. The resulting mixtures were stirred at 0-5°C for at least 10 minutes until the coloured solids were formed. The resulting precipitates formed were filtered, washed with cold water and recrystallized with hot chloroform to yield the pyridine azo compounds. The purity of the compounds were detected using Thin Layer Chromatography (TLC).



3-((2,4-dimethylphenyl)diazenyl)pyridine



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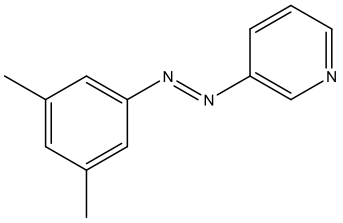

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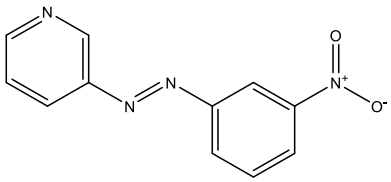

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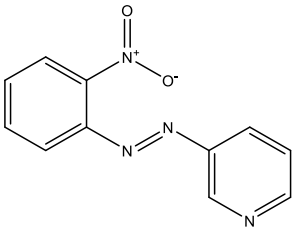
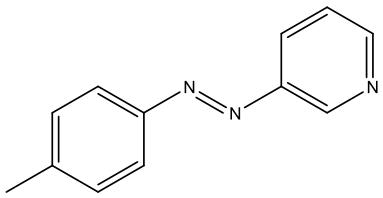


3-((2-nitrophenyl)diazenyl)pyridine

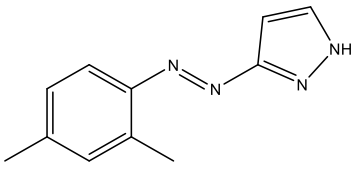


3-(p-tolyldiazenyl)pyridine

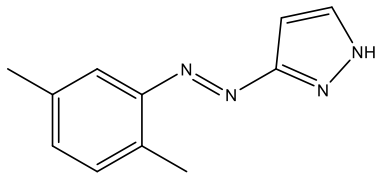
Fig. 1. Structure of Pyridine azo derivatives

B. Synthesis Of Pyrazole Azo Compounds

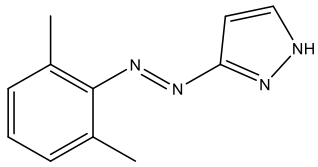
The pyrazole derivatives were synthesized by diazotization of amines followed by coupling with pyrazole as described earlier. (ref) In brief, solutions of aromatic amines (10 mmol) were prepared by dissolving 2,4-Dimethylaniline (29mg), 2,5-Dimethylaniline (29mg), 2,6-Dimethylaniline (29mg), 3,5-Dimethylaniline (29mg), N,N-Dimethylaniline (29mg), Para Toluidine (25.7mg) and Para Chloroaniline (25.7mg) in 6 ml of 3M HCl and 8 ml of water individually. The mixtures were cooled to 0°C in an ice bath with stirring. Once this solution was cooled to 0-5°C, 10 ml of freshly prepared 1M sodium nitrite solution was added slowly, maintaining the temperature below 5°C. The solution was kept in an ice bath. 10 mmol pyrazole solution was prepared by dissolving 47.5 mg of pyrazole in 60 ml of 2M sodium hydroxide, and cooled to 0-5°C in an ice bath. 10ml of this solution was slowly added to the cooled substituted diazonium chloride solutions of aromatic amines. The resulting mixtures were stirred at 0-5°C for at least 10 minutes until the coloured solids were formed. The resulting precipitates formed were filtered, washed with cold water and recrystallized with hot chloroform to yield the pyrazole azo compounds. The purity of the compounds were detected using Thin Layer Chromatography (TLC).



3-((2,4-dimethylphenyl)diazenyl)-1H-pyrazole



3-((2,5-dimethylphenyl)diazenyl)-1H-pyrazole



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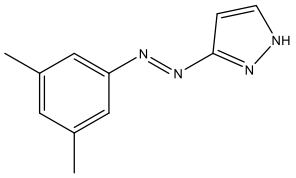
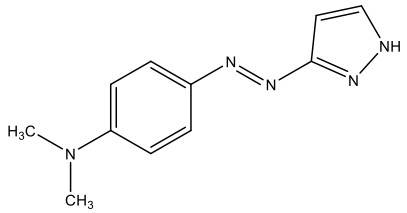
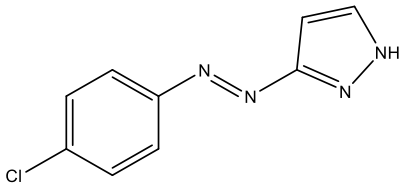


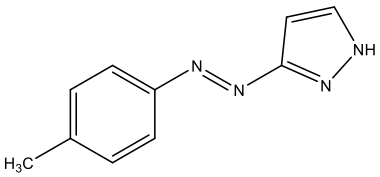
3-((3,5-dimethylphenyl)diazenyl)-1H-pyrazole



4-((1H-pyrazol-3-yl)methyl)diazenyl)-N,N-dimethylaniline



3-((4-chlorophenyl)diazenyl)-1H-pyrazole



3-(p-tolyldiazenyl)-1H-pyrazole

Fig.2. Structure of Pyrazole azo derivatives

C. Physiochemical characterization

The UV spectrophotometric determination was carried out with a Shimadzu UV-1800 spectrophotometer. In brief, 1mg of pyridine and pyrazole azo compounds were dissolved in 1ml of chloroform and transferred to 96 well plate and read from 200 to 800 nm to determine the absorption maxima of the compounds. (Optical path length 1.000 or 0.100 cm). The KBr pellets of pyridine and pyrazole azo compounds were prepared and mounted on IR path in 470-Shimadzu Infrared Spectrophotometer. The FTIR spectrum was recorded in the region 4000-400 cm-1.The synthesized compounds were dissolved in deuterated chloroform and the ^{1}H-NMR and ^{13}C-NMR spectra were recorded in ppm units using BrukerAvance II 400 NMR Spectrometer (Billerica, MA, USA) at400 MHz Frequency.

D. Antibacterial activity

To evaluate the antimicrobial efficacy of the newly synthesized pyridine and pyrazole compounds in vitro, initially they were screened against gram positive (Staphylococcus aureus, Enterococcus faecalis) and gram negative (Klebsialla pneumonia and Escherichia coli) bacterial, yeast (Candida albicans) and fungal (Aspergillus favus) pathogens using disc diffusion test. In brief, the overnight cultures of bacteria (grown in nutrient broth) and yeast (grown in sabourad dextrose broth) and a week culture of fungal spores (grown in potato dextrose broth) were spread on the Muller Hinton agar plates. The pyridine and pyrazole azo compounds were dissolved in DMSO (1mg/ml) and loaded on the blank discs. The discs were air dried in the laminar airflow chamber and the dried discs were placed on the plates containing test microbes. Then the plates were incubated for 24 h at 37°C for bacteria and 48h at 28°C for yeast and fungi. Disc loaded with DMSO alone was used as negative control.Streptomycin (25 mg/mL) and Fluconazole (25 mg/mL) were used as standard for bacteria and fungi respectively. Antimicrobial activities were expressed as follows based on the zones of inhibition (in mm): NA-no activity); less than 4 mm-weak;5-9 mm-moderate and greater than 10mm-strong.The minimum inhibitory concentration (MIC) of pyridine and pyrazole azo compounds those had strong zone of inhibition in the disc diffusion test was determined by serial double dilution method. The compounds were dissolved in DMSO (10 mg/ml) stock solutions. 0.2ml of stock test solution was added in to 4.8ml of Muller Hinton broth. 2.5ml of this solution was added into 2.5ml of broth followed by six more similar serial dilution. The bacterial and fungal cultures were adjusted to 10^{4}-10^{5}cfu/ml for bacteria and 10^{2}cfu/ml for fungi and added to each tubes. The tubes with 0.1ml of DMSO alone was used as control. All the tubes were incubated at 24 h at 37°C for bacteria and 48h at 28°C for yeast and fungi.

Table 1: Antimicrobial activity of Pyridine derived azo dyes –Disc diffusion study (Zone of inhibition in mm; PC-Positive control: Streptomycin for bacteria and fluconazole for fungus)

Sample Code	Staphylococcus aureus (G+) MTCC 916	Enterococcus faecalis (G+)	Klebsiella pneumonia (G-) MTCC 503	E.coli (G-) MTCC 1671	Candida albicans (F) MTCC 277	Aspergillus flavus (F)
2,4-Pyr	8	7	8	7	8	-
2,5-Pyr	8	7	8	7	7	-
3,5-Pyr	7	8	7	8	9	-
O,N-Pyr	7	7	10	10	7	-
M,N-Pyr	8	10	10	8	9	-
P,T-Pyr	7	7	10	7	7	-
PC	17	21	22	17	17	20

Table 2: Minimal inhibitory concentration of Pyridine derived azo dyes (in µg/ml)

Sample Code	Staphylococcus aureus (G+) MTCC 916	Enterococcus faecalis (G+)	Klebsiella pneumonia (G-) MTCC 503	E.coli (G-) MTCC 1671	Candida albicans (F) MTCC 277	Aspergillus flavus (F)
2,4-Pyr	50	50	50	50	50	-
2,5-Pyr	50	50	50	50	50	-
3,5-Pyr	50	50	25	50	25	-
O,N-Pyr	50	50	25	25	50	-
M,N-Pyr	50	25	25	50	25	-
P,T-Pyr	50	50	25	50	50	-

Table 3: Antimicrobial activity of Pyrazole derived azo dyes –Disc diffusion study (Zone of inhibition in mm; PC-Positive control: Streptomycin for bacteria and fluconazole for fungus)

Sample Code	Staphylococcus aureus (G+) MTCC 916	Enterococcus faecalis (G+)	Klebsiella pneumonia (G-) MTCC 503	E.coli (G-) MTCC 1671	Candida albicans (F) MTCC 277	Aspergillus flavus (F)
2,4-PZ	11	11	9	11	7	-
2,5-PZ	8	-	19	7	12	-
2,6-PZ	16	14	13	15	12	9
3,5-PZ	18	12	10	10	8	8
N,N-PZ	12	11	12	9	9	-
P,Cl-PZ	9	10	9	9	10	11
P,T-PZ	8	13	9	10	7	8
PC	15	19	20	17	14	24

Table 4: Minimal inhibitory concentration of Pyrazole derived azo dyes (in µg/ml)

Sample Code	Staphylococcus aureus (G+) MTCC 916	Enterococcus faecalis (G+)	Klebsiella pneumonia (G-) MTCC 503	E.coli (G-) MTCC 1671	Candida albicans (F) MTCC 277	Aspergillus flavus (F)
2,4-PZ	12.5	12.5	25	12.5	50	-
2,5-PZ	50	-	6.25	50	12.5	-
2,6-PZ	6.25	6.25	12.5	6.25	12.5	25
3,5-PZ	6.25	12.5	25	25	50	50
N,N-PZ	12.5	12.5	12.5	25	25	-
P,Cl-PZ	25	25	25	25	25	12.5
P,T-PZ	12.5	12.5	12.5	12.5	12.5	25

E. Antioxidant activity

Free radical scavenging activity of pyridine and pyrazole azo derivatives was determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH) assay according to the method of Sanchez-Moreno(Sanchez-Moreno, 2002). In brief, 10 mg/ml solutions of all synthesized azo-compounds were prepared and double diluted up to 0.01mg/ml. 50 µl of each compound was added into the 2 ml of freshly prepared 0.2 mM DPPH solution in methanol and incubated for 20 min at 37 °C. After incubation, the absorbance was measured at 517 nm using spectrophotometer. Similar concentrations of ascorbic acid were used as a positive control for comparison. The percentage scavenging of DPPH radical of the synthesized azo-derivatives was calculated by using the following formula.

% Radical Scavenging Activity = [(Ac -As)/Ac] x100

Where 'Ac' is the absorbance of the control sample (DPPH solution without sample) and 'As' is the absorbance of the sample (DPPH solution + Azo compound). Test was performed in triplicate.

Table 5: Percentage inhibition of DPPH at different concentrations of Pyridine and Pyrazole azo dyes

Sample name	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	0.62 mg/ml	0.31 mg/ml	0.15 mg/ml	0.07 mg/ml	0.03 mg/ml	0.01 mg/ml
	% inhibition									
Standard (Ascorbic acid)	61.2±5.9	39.4±2.3	30.4±1.8	21.3±1.8	15.2±2.1	9.1±0.8	7.1±0.3	6.1±.3	4±0.6	3±0.5
2,4-Pyr	39.4±3.4	26.6±3.2	15.3±3.2	7.5±1.4	5.6±0.7	3.4±0.8	2±0.4	2.1±0.2	2±0.3	2±0.4
2,5-Pyr	40.4±3.1	20.5±1.2	17.2±2.3	9.7±1.1	6.3±0.6	4.2±0.8	2±0.9	2±0.7	2±0.5	2±0.2
3,5-Pyr	35.4±5.1	17.7±4.3	10.7±6.3	5.7±3.4	4.1±2.3	3.1±1.8	2±0.1	2±0.3	2±0.3	2±0.5
O,N-Pyr	51.2±3.4	23.2±4.3	18.2±1.3	15.3±1.1	10.1±1.4	7.3±0.8	4.9±0.4	2.2±0.4	2±0.8	2±0.7
M,N-Pyr	54.3±2.4	26.1±2.3	20.6±1.3	16.6±2.2	11.9±1.7	7.7±1.1	4.7±0.7	2.4±0.4	2±0.4	2±0.5
P,T-Pyr	49.4±3.5	25.6±4.3	16.4±3.2	9.8±4.3	6.8±2.3	4.1±1.7	3.2±0.4	2.2±0.4	2±0.6	2±0.1
2,4-PZ	33.4±4.5	16.2±3.1	8.4±2.5	4.9±2.1	3.2±0.8	2.1±1.1	2±0.7	2±1.1	2±0.8	2±0.6
2,5-PZ	32.3±2.3	14.2±2.3	9.5±2.7	5.5±2.2	4.3±1.7	3.2±0.9	2.2±0.4	2±0.8	2.1±0.6	2±0.1
2,6-PZ	31.9±3.6	15.4±3.5	8.3±4.6	5.2±4.4	3.6±2.1	3±1.8	2.4±0.6	2.1±0.2	2±0.3	2±0.3
3,5-PZ	32.6±3.4	18.8±3.9	11.7±5.2	7.7±2.6	4.9±0.9	2.9±1.8	2±0.5	2±0.7	2.2±0.5	2±0.4
N,N-PZ	47.2±2.3	22.1±1.4	15.6±2.5	9.1±3.2	5.9±1.5	3.4±1.7	2.2±0.5	2±0.4	2±0.7	2±0.2
P,Cl-PZ	30.6±5.1	17.9±2.3	12.4±3.5	7.2±2.1	2.7±1.8	2.4±1.1	2±0.2	2±0.4	2.2±0.4	2±0.2
P,T-PZ	57.3±3.6	32.2±5.2	24.7±2.7	18.8±3.4	13.7±2.1	9.4±2.1	7.7±2.6	4.4±1.2	2.3±0.2	2±0.3

III. Result and Discussion

A. Synthesis and characterization

Total of six pyridine and seven pyrazole derived azo compounds were synthesized, those had better yield and characterized. The compounds were named based on the structural nomenclature and each compound characteristics are given below;

B. Pyridine Azo derivatives

1. 3-((2,4-dimethylphenyl)diazenyl)pyridine: Yield: 68%,UV spectrum analysis showed peak (λmax) at 452 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 8.70 (m, 1H, H2-Pyridine), 7.54 (m, 1H, H3-Pyridine), 8.42 (d, 1H, H4-Pyridine), 8.98 (d, 1H, H6-Pyridine), 7.06 to 7.14 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 18.1, 21.6, 122, 125, 126, 128, 128.6, 130.2, 130.9, 136.9, 145.1, 147.6, 151.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2969 (C-H of R-CH_3), 2865 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).
2. 3-((2,5-dimethylphenyl)diazenyl)pyridine: Yield: 70%,UV spectrum analysis showed peak (λmax)at 361 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 8.70 (m, 1H, H2-Pyridine), 7.54 (m, 1H, H3-Pyridine), 8.42 (d, 1H, H4-Pyridine), 8.98 (d, 1H, H6-Pyridine), 7.06 to 7.14 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 17.8, 21.3, 122.8, 127.9, 128, 128.9, 130.2, 130.6, 134, 135.4, 145.1, 147.6, 151.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2969 (C-H of R-CH_3), 2865 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).

3. 3-((3,5-dimethylphenyl)diazenyl)pyridine: Yield: 72%,UV spectrum analysis showed peak (λmax) at 462 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 8.70 (m, 1H, H2-Pyridine), 7.54 (m, 1H, H3-Pyridine), 8.42 (d, 1H, H4-Pyridine), 8.98 (d, 1H, H6-Pyridine), 7.06 to 7.14 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 21.6, 21.6, 122.8, 125.8, 125.8, 128, 128.5, 130.9, 138.3, 138.3, 145.1, 147.6, 151.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2969 (C-H of R-CH_3), 2865 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1590 (C=C of aromatic).
4. 3-((3-nitrophenyl)diazenyl)pyridine: Yield: 75%,UV spectrum analysis showed peak (λmax) at 391nm. ^{1}H NMR ($CHCl_3$-d6) d 8.70 (m, 1H, H2-Pyridine), 7.54 (m, 1H, H3-Pyridine), 8.42 (d, 1H, H4-Pyridine), 8.98 (d, 1H, H6-Pyridine), 7.50 to 7.90 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 122.8, 123.4, 123.9, 128, 129.6, 129.7, 134.8, 145.1, 147.6, 149.7, 151.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 760-780 (C-H of aromatic), 1600 (C=C of aromatic).
5. 3-((2-nitrophenyl)diazenyl)pyridine: Yield: 69%,UV spectrum analysis showed peak (λmax) at 445 nm. ^{1}H NMR ($CHCl_3$-d6) d 8.70 (m, 1H, H2-Pyridine), 7.54 (m, 1H, H3-Pyridine), 8.42 (d, 1H, H4-Pyridine), 8.98 (d, 1H, H6-Pyridine), 7.50 to 7.90 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 122.8, 123.1, 123.9, 128, 128.4, 131, 134.8, 145.1, 147.6, 147.9, 151.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 760-780 (C-H of aromatic), 1600 (C=C of aromatic).
6. 3-(p-tolyldiazenyl)pyridine: Yield: 80%,UV spectrum analysis showed peak (λmax) at 401 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.34 (s, 3H, CH_3), 8.70 (m, 1H, H2-Pyridine), 7.54 (m, 1H, H3-Pyridine), 8.42 (d, 1H, H4-Pyridine), 8.98 (d, 1H, H6-Pyridine), 7.50 to 7.90 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d21.3, 122.8, 125.7, 128, 128.7, 128.7, 129, 129, 138.4, 145.1, 147.6, 151.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2969 (C-H of R-CH_3), 2865 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1600 (C=C of aromatic).

C. Pyrazole Azo derivatives

1. 3-((2,4-dimethylphenyl)diazenyl)-1H-pyrazole: Yield: 78%,UV spectrum analysis showed peak (λmax)at 463 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.48 (m, 1H, H2-Pyrazole), 7.89 (m, 1H, H3-Pyrazole), 12.51 (d, 1H, NH-Pyrazole), 7.06 to 7.14 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 18.1, 21.6, 95.4, 125, 126, 128.6, 130.2, 130.9, 131.7, 136.9, 146.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2960 (C-H of R-CH_3), 2870 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).
2. 3-((2,5-dimethylphenyl)diazenyl)-1H-pyrazole: Yield: 68%,UV spectrum analysis showed peak (λmax) at 420 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.48 (m, 1H, H2-Pyrazole), 7.89 (m, 1H, H3-Pyrazole), 12.51 (d, 1H, NH-Pyrazole), 7.06 to 7.14 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 17.8, 21.3, 95.4, 127.9, 128.9, 130.2, 130.6, 131.7, 134, 135.4, 136.9, 146.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2960 (C-H of R-CH_3), 2870 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).
3. 3-((2,6-dimethylphenyl)diazenyl)-1H-pyrazole: Yield: 72%,UV spectrum analysis showed peak (λmax)at 400 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.48 (m, 1H, H2-Pyrazole), 7.89 (m, 1H, H3-Pyrazole), 12.51 (d, 1H, NH-Pyrazole), 7.06 to 7.14 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 18.1, 18.1, 95.4, 127, 128.5, 128.8, 128.8, 131.7, 136.9, 136.9, 146.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2960 (C-H of R-CH_3), 2870 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).
4. 3-((3,5-dimethylphenyl)diazenyl)-1H-pyrazole: Yield: 74%,UV spectrum analysis showed peak (λmax)at 462 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.28 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 6.48 (m, 1H, H2-Pyrazole), 7.89 (m, 1H, H3-Pyrazole), 12.51 (d, 1H, NH-Pyrazole), 7.06 to 7.14 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 21.6, 21.6, 95.4, 125.8, 125.8, 128.5, 130.9, 131.7, 138.3, 138.3, 146.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2960 (C-H of R-CH_3), 2870 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).
5. 4-((1H-pyrazol-3-yl)methyl)diazenyl)-N,N-dimethylaniline: Yield: 70%,UV spectrum analysis showed peak (λmax) at 389 nm. ^{1}H NMR ($CHCl_3$-d6) d 3.02 (s, 3H, CH_3), 3.02 (s, 3H, CH_3), 6.48 (m, 1H, H2-Pyrazole), 7.89 (m, 1H, H3-Pyrazole), 12.51 (d, 1H, NH-Pyrazole), 6.83 to 7.74 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 41.3, 41.3, 95.4, 111.4, 111.4, 130.9, 130.9, 131.7, 146.8, 151.1, 154.3. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2960 (C-H of R-CH_3), 1330 (C-N of amine), 1280-1320 (C-N of Ph-$(R)_2$), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).
6. 3-((4-chlorophenyl)diazenyl)-1H-pyrazole: Yield: 78%,UV spectrum analysis showed peak (λmax) at 403 nm. ^{1}H NMR ($CHCl_3$-d6) d 6.48 (m, 1H, H2-Pyrazole), 7.89 (m, 1H, H3-Pyrazole), 12.51 (d, 1H, NH-Pyrazole), 6.83 to 7.74 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 95.4, 126.8, 128.8, 128.8, 130.2, 130.2, 131.7, 134.3, 146.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 760-780 (C-H of aromatic), 700 (C-Cl), 1660 (C=C of aromatic).
7. 3-(p-tolyldiazenyl)-1H-pyrazole:Yield: 80%,UV spectrum analysis showed peak (λmax) at 443 nm. ^{1}H NMR ($CHCl_3$-d6) d 2.34 (s, 3H, CH_3), 6.48 (m, 1H, H2-Pyrazole), 7.89 (m, 1H, H3-Pyrazole), 12.51 (d, 1H, NH-Pyrazole), 7.40 to 7.79 (s, 1H, benzene-CH); ^{13}C NMR ($CHCl_3$-d6) d 21.3, 95.4, 125.7, 128.7, 128.7, 129, 129, 131.7, 138.4, 146.8. FTIR: (KBr, cm^{-1}) 1550 (N=N), 2960 (C-H of R-CH_3), 2870 (C-H of Ph-CH_3), 760-780 (C-H of aromatic), 1660 (C=C of aromatic).

D. Antimicrobial activity

The antibacterial and antifungal activity of pyridine and pyrazole derived azo compounds are showed in table 1 to 4. The disc diffusion study showed moderate to strong antibacterial activity against Staphylococcus aureus, Enterococcus faecalis, Klebesiella pneumoniae and Escherichia coli by the synthesized pyridine azo derivatives. They also showed effective inhibition of Candida albicans whereas none of the compounds were effective against fungus Aspergillus flavus. (Table1). Table 2 shows the minimum inhibitory concentration of the compounds. From all the compounds, the M,N-Pyr was more effective on Enterococcus faecalis. 3,5-Pyr, M,N-Pyr, O,N-Pyr and P,T-Pyr were exhibited significant activity against Klebesiella pneumoniae. The 3,5-Pyr and M,N-Pyr were found be efficient against Candida albicans compared to others.

All the pyrazole derived azo compounds showed against all tested bacteria and Candida albicans except the 2,5-PZ that was not active against Enterococcus faecalis. The compounds, 2,6-PZ, 3,5-PZ, P,Cl-PZ and P,T-PZ were showed significant activity against Aspergillus flavus whereas 2,4-PZ, 2,5-PZ and N,N-PZ were not effective (Table 3). From this study, we found that 2,6-PZ was more efficient compound followed by P,Cl-PZ and P,T-PZ against tested bacterial and fungal pathogens (Table 4). Modern era aims to develop pharmacological agents against resistant bacteria to minimize infection related morbidity and mortality. For example, form 19th century, the Gram-positive organisms have developed resistance against the action of β-1actams and other antimicrobials among *Enterococcus faecium*strains easily transmit genetic material to more virulent species such as the methicillin resistant *S.aureus.* Additionally, *Klebsiella pneumonia* and *Escherichia coli produce enzymes that inactivate* newer cephalosporins. (Ref) Various studies showed that the pyridine, pyrazole and pyrazoline derivatives have antimicrobial activities(Aldridge, Gelfand, Schiro, & Barg, 1992; Jacoby & Carreras, 1990; Moellering, 1992). It was observed that the compounds with heterocyclic rings showed antimicrobial activity compared with non-hetercyclic ring. In this study we found that the addition of heterocyclic rings to both pyridine and pyrazole might enhanced their antimicrobial activities. In addition, the presence of ortho and metanitroaniline in the aromatic ring with pyridine showed enhanced activity might be due to active reactive oxygen moiety. In comparison with pyridine azo derivatives, pyrazole azo derivatives showed significant antimicrobial activity.

E. Antioxidant activity

Oxidative stress leads to the formation of free radicals which can damage cellular organelles including cell membrane and nucleic acids.Previous studies showed that the heterocyclic compounds possess the antioxidant activities(Abrigach et al., 2014). In this study, we calculated the DPPH scavenging activity of the newly synthesized pyridine and pyrazole azo compounds in addition to the control ascorbic acid, a known antioxidant (Table 5). We found that all the compounds possess the antioxidant activity at higher concentration (10mg/ml), whereas P,T-PZ showed significant (57.3±3.6) free radical scavenging activity followed by O,N-Pyr (51.2±3.4) and M,N-Pyr (54.3±2.4). It has been observed that the phenylaryl compounds and aryl compounds possess methyl groups at different position have minimum to maximum antioxidant potential (Selvam, Jachak, Thilagavathi, & Chakraborti, 2005). The molecules with N-heterocyclic moieties have potential antioxidant efficacy and the nitrogen present in the newly synthesized pyridine and pyrazole compounds might enhanced the absorbance of free radicals.

IV. Conclusion

In this study, we synthesized new heterocyclic pyridine and pyrazole azo derivatives and characterized them. We found that majority of these azo derivatives has the antimicrobial and antioxidant potential, which can be used in the discovery of new pharmaceutical agents for treating infectious diseases. Further evaluation of molecular mechanism, how these compounds act on the bacteria and fungi will elucidate their specific role and leads to develop new drugs especially for the drug resistant pathogens.

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