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Biological Study of Some Newly Photo Induced Acridinium Catalyzed, Synthesized [8] Spiro - Cyclic Pyrazolone Derivatives

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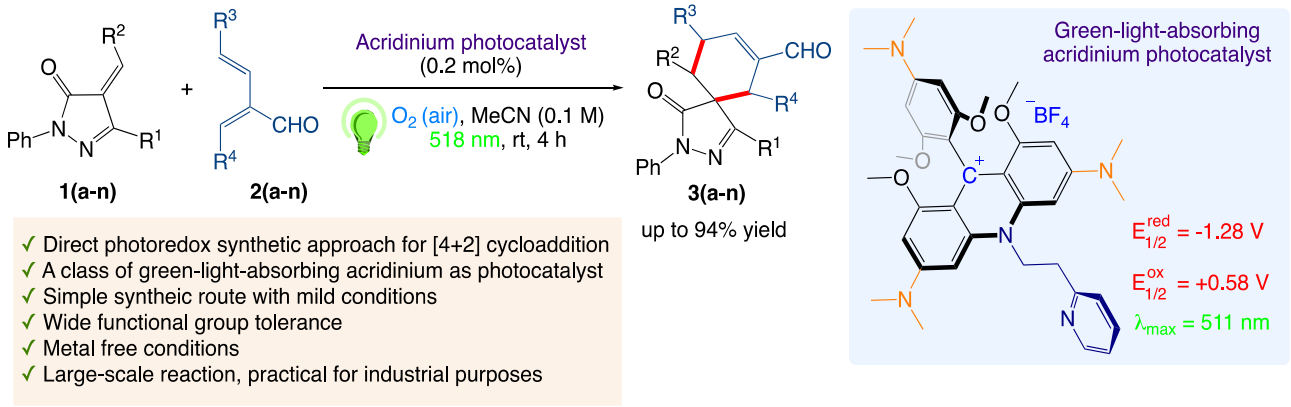
Abstract: ***The objective of this study was evaluation of biological activity of (3a - 3n) spiro - pyrazolones, synthesized by visible light mediated organo - photo redox acridinium catalysis. This examination by single spore isolation and disc diffusion method shows that all compounds have antimicrobial activity. The compounds 3l, 3m shows best antibacterial activity against Gram positive bacteria Staphylococcus aureus, Bacillus subtilis, Gram negative bacteria Pseudomonas aeruginosa, Escherichia coli and antifungal activity against Aspergillus niger.***

Keywords: Spiro - pyrazolones, Antibacterial activity, Inhibition zones, Single spore isolation, Disc diffusion

1. Introduction

Spiro - Pyrazolones are well known biologically active molecules, have and good anti - microbial activity [1]. These are important for their analgesics [2], hypoglycemic activity [3]. Many Pyrazolone scaffolds are used as fungicides [4, 5]. Spiro derivatives having their natural abundance, provides more attention for their applications [7]. In our previous work, we have synthesized spiro - cyclic pyrazolone 3 (a - n) derivatives by using following scheme.



Scheme 1: Visible - light - induced organo photoredox - catalyzed synthesis of bioactive spiro - cyclic pyrazolones

It was a novel organo catalytic synthetic protocol, has been developed here to synthesize bioactive spiro - cyclic pyrazolones. The potential utility of this reaction was newly developed metal free photoredox protocol for [4+2] cycloaddition is broad in scope and tolerant of a variety of functional groups. The synthesis of various economically significant building blocks for a wide range of bioactive and pharmacological substances serves as an important illustration of its value. On the basis of adaptability and operational simplicity of this reaction, we anticipate that this method will provide an innovative and sustainable reaction platform to achieve a robust and practical synthetic utility.

Now our group is proceeding forward for biological study of these newly synthesized spiro - cyclic pyrazolone 3 (a - n). ***Biological evaluation of all our synthesized 3 (a - n) compounds [8] has been carried out by disc diffusion method [9].*** The microbial colony of Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa, Escherichia coli has been taken for antibacterial and Aspergillus niger for antifungal evaluation of spiro - cyclic pyrazolones. **Staphylococcus aureus** causes infections in human and other animals [10, 11]. **Bacillus subtilis**, naturally transformable, gram positive, fast growing, aerobic, rod shaped bacteria, easy to cultivate. The endo - spores produced by Bacillus subtilis provide fruitful system for studying biofilms [12]. **Pseudomonas aeruginosa**, is an important pathogen, infect hospitalized peoples and other cystic fibrosis patients [13]. **Escherichia coli** is rod shaped, 2.0 µm long, coli form bacterium [14, 15], and found in lower intestine of warm - blooded organism [16, 17]. **Aspergillus niger** 'black mould' causes infections on onions, grapes and other many fruits, having 3 - 5 µm of spherical conidia, Isolated from seeds of Soybean, Maize, Gram seeds [18].

2. Experimental

Compounds (3a - 3n) were evaluated in vitro using the Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) disc diffusion methods, N, N - di methyl formamide as solvent. Nutrient Agar medium (NA) was used for bacterial sub - culture, whereas, and Sabouraud Dextrose Agar (SDA) for fungal sub - culture. Petri plates were prepared by pouring 50 mL of NA or SDA and allowing it to solidify. All microbial species were isolated from infected part of hosts.

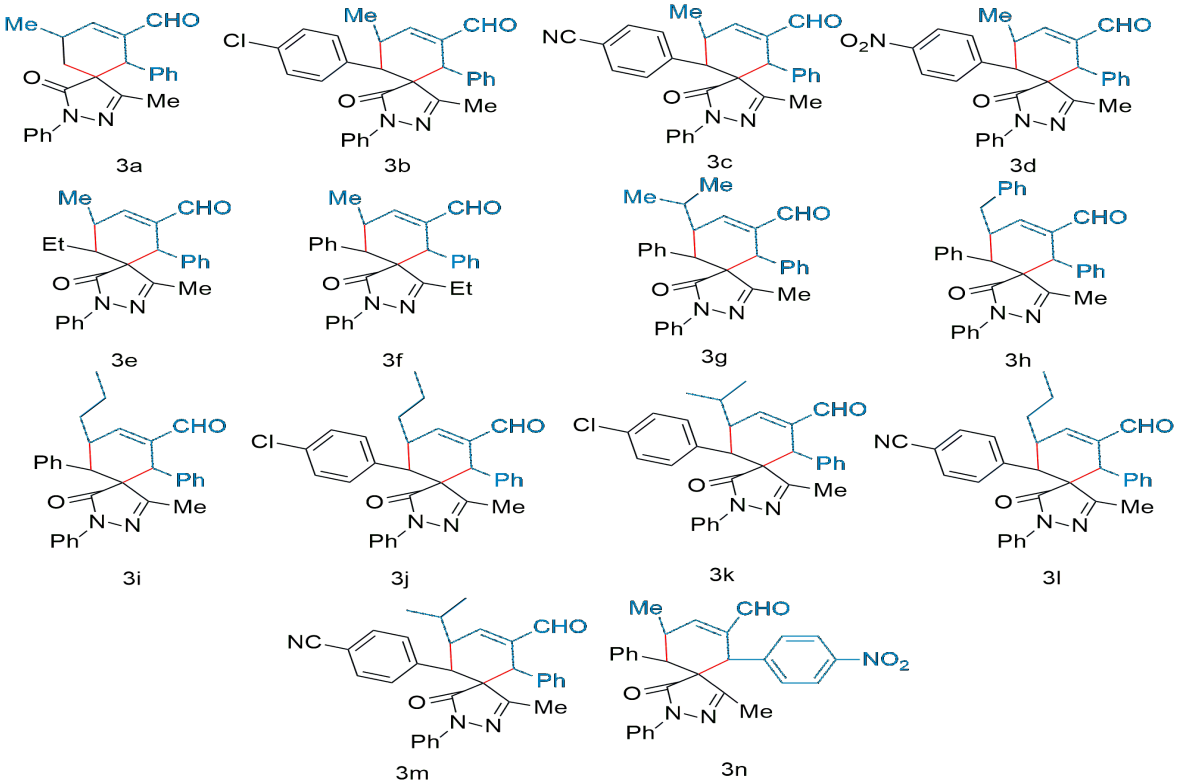
Inoculums preparation: From the above bacterial culture, 4 to 5 colonies are transferred in 5 mL 0.9% saline with the help of wire loop, incubated at 30^0 C, when it exceeds turbidity of 0.5 Mac Farland standards, reduce the turbidity by adding same saline. Plates were dried and 1 mL of each standardized inoculums suspension was poured and uniformly spread. Amphotericine - B, Ciprofloxacin, and Amoxicillin, were used as standard compound as antibacterial and antifungal agents, DMF alone showed no inhibition zone. The plates were incubated at 37 °C for 24 h. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth around the disks in mm.

3. Results and discussions

Our group has been synthesized bioactive pyrazolones 3 (a - n) by visible light induced acridinium catalysis [8], and characterization of product was done by ^{1}H NMR and ^{13}C NMR. The spectra were recorded on Bruker AVANCE DPX (400 MHz and 100 MHz) FT spectrometer in $CDCl_3$ using TMS as an internal reference (chemical shift in ϭ ppm). In our aim for biological study of synthesized compounds, we have done evaluation of antibacterial, antifungal activity against Gram positive and Gram negative bacteria and fungi.

In our study we find that all are biologically active compounds. Some of them (**3b, 3d, 3l, 3m, 3i)** are considerably active against bacteria and (**3c, 3j, 3l, 3m)** are active against fungi.

Table 1: Our synthesized compounds by organo photo redox acridinium catalysis



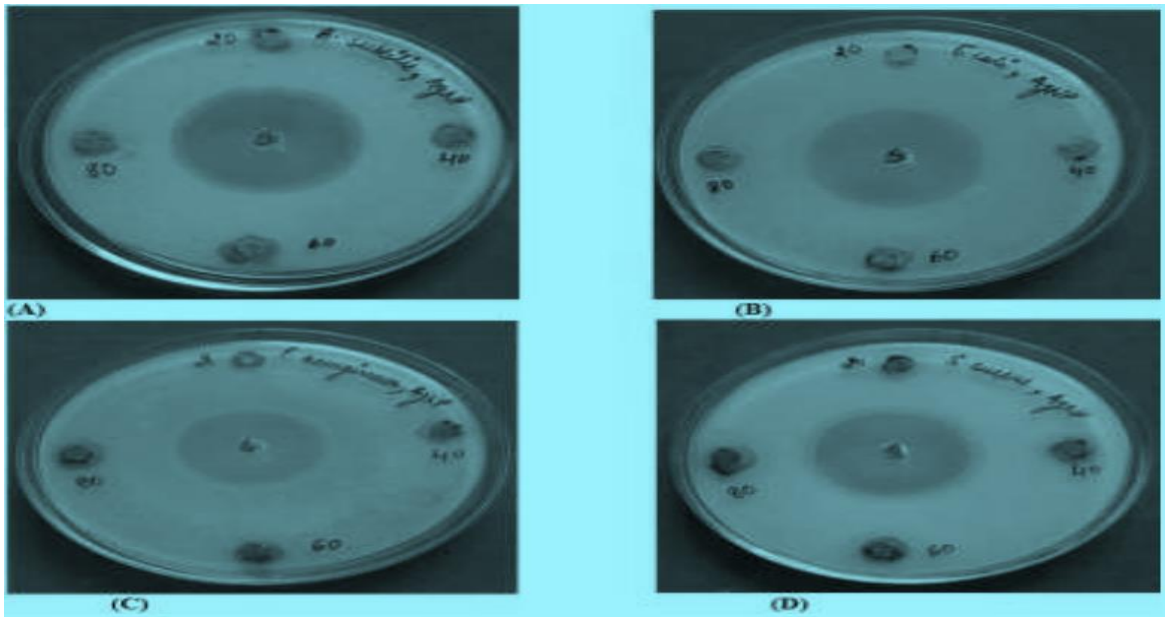


Figure 1: Antibacterial efficiency of **3l** against Gram - positive Staphylococcus aureus and Bacillus subtilis (A and B) and gram - negative bacteria Escherichia coli and Pseudomonas aeruginosa (C and D) respectively

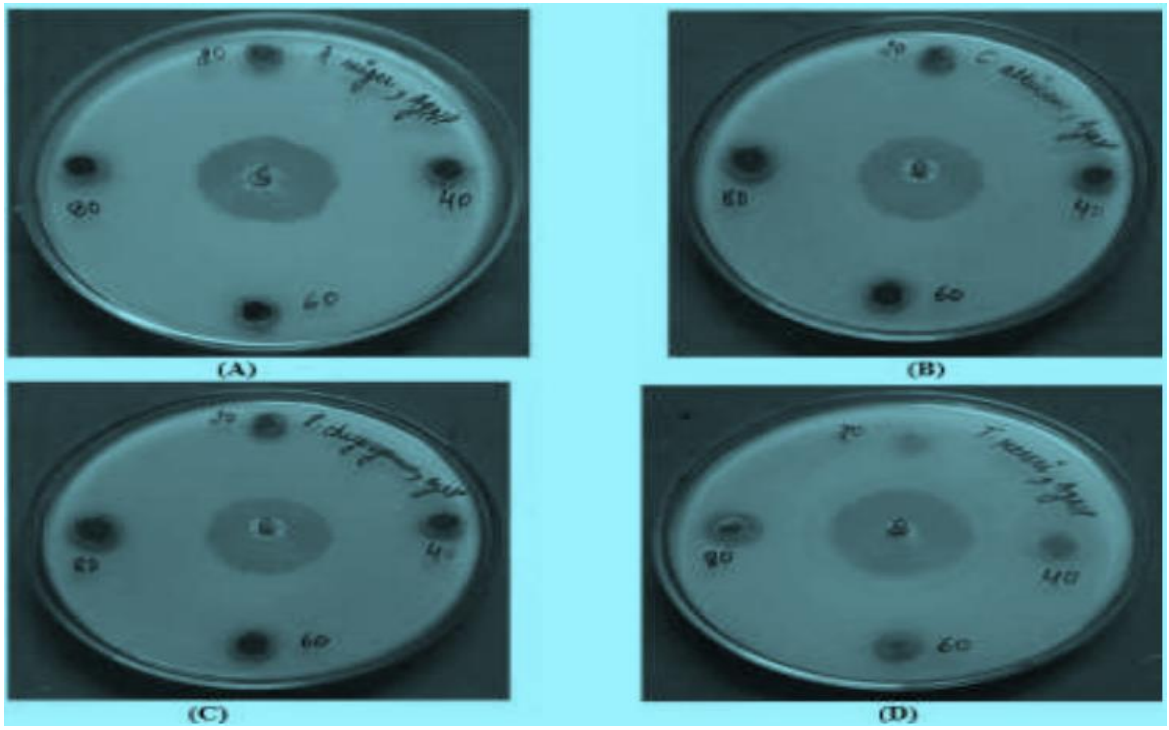


Figure 2: Antifungal efficiency of (**3c, 2j, 3l, 3m)** against Aspergillus niger **A, B, C, D** respectively.

4. Biological Activity

Since pyrazolone derivatives are well known for their biological activity. These have good antipyretic, antitubercular, antiarthritic, anti - inflammatory and antitumor activities. Here we are interested for the study of antimicrobial properties of our synthesized compounds. The bactericidal and fungicidal activity of all 3 **(a - n)** synthesized Spiro - cyclic pyrazolones has been screened and data is given in Table - 2 & 3.

4.1 Antibacterial activity

The evaluation of compounds 3 **(a - n)** shows that the molecule **3l** and **3m** shows best antibacterial activity against all four bacterium having inhibition zone nearest to their reference drugs, due to presence of p - CN C_6H_5 group neighboring to propyl and isopropyl group and cyanide - carbonyl group interaction. The compound **3i** is highly active against **S. aureus and B. subtilis** having inhibition zone 20, 25 mm near to that of Amoxicillin due to presence of carbonyl oxygen between two phenyl groups and methyl phenyl interactions. The compound **3d** is active against **S. aureus** with inhibition zone 22 mm but less active against **B. subtilis** having carbonyl oxygen hindered by phenyl group and p - nitro phenyl groups. The compound **3d** also moderately active against both gram negative bacterium with inhibition zone 23 and 28 mm more closed to reference drug ciprofloxacin of 30.2 and 25.8 mm. The compound **3b** is also highly active against **B. subtilis** with inhibition zone

27 mm close to reference drugs amoxicillin having inhibition zone 28.3 mm. Other compounds are also active against bacteria, with moderate to low range.

Table 2: Data of antibacterial activity for Spiro - cyclic Pyrazolones

S. N.	Spiro – Pyrazolones	Gram (+) Ve, Bacteria*		Gram (-) Ve, Bacteria*	
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
1	3a	18	18	16	18
2	3b	10	27	12	8
3	3c	11	9	9	6
4	3d	22	15	28	23
5	3e	5	8	15	10
6	3f	16	14	9	18
7	3g	10	14	13	12
8	3h	8	9	15	9
9	3i	20	25	12	17
10	3j	13	15	20	9
11	3k	18	15	12	12
12	3l	22	25	28	24
13	3m	23	27	27	23
14	3n	18	15	15	12
X	Ciprofloxacin	-	-	30.2	25.8
Y	Amoxicillin	25.6	28.3	-	-

*Inhibition zone in mm of Spiro - Pyrazolones and reference drugs for bacteria

4.2 Antifungal activity

The evaluation of compounds **3 (a - n)** shows that the compounds **3c, 3j, 3l, 3m** are highly active against **A. niger** with inhibition zone 23, 21, 25, 24 near to reference drugs Amphotericine - B having that of 26.8 mm, due to presence of carbonyl oxygen between p - CN phenyl and phenyl groups. Other compounds also show antifungal activity with moderate to low range.

Table 3: Data of antifungal activity for Spiro - cyclic Pyrazolones

S. N.	Spiro - Pyrazolones	Fungi*
		Aspergillus *nigar*
1	3a	10
2	3b	10
3	3c	23
4	3d	13
5	3e	12
6	3f	16
7	3g	10
8	3h	9
9	3i	6
10	3j	21
11	3k	14
12	3l	25
13	3m	24
14	3n	11
X	Amphotericine - B	26.8

*Inhibition zone in mm of Spiro - Pyrazolones and reference drugs for fungi

5. Conclusions

Here we have done the evaluation of our synthesized all 3 **(a - n)** compounds for their anti - bacterial properties against Gram - positive bacteria Staphylococcus aureus, Bacillus subtilis and Gram - negative bacteria Pseudomonas aeruginosa, Escherichia coli and antifungal properties against fungus Aspergillus niger by disc diffusion method. All are active against these micro - organisms.

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