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SYNTHESIS OF NEW ANTHRANILIC ACID DYES AND EVALUATION FOR THEIR ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITIES

P.D. Gaikwad*, V.H. Bankar, S. B. Bodele and A. P. Mehere
Sharad Pawar College of Pharmacy, Wanadongri, Nagpur - 441 110 (M.S.) India.
E-mail: *preeti_gaikwad2002@yahoo.com*

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ABSTRACT

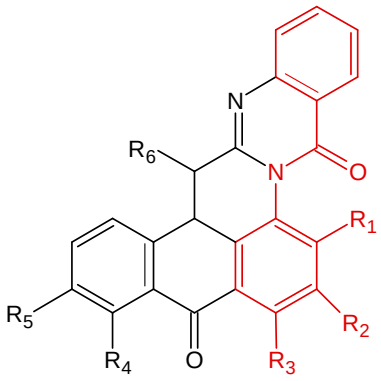
A new series of dyes were synthesized from anthranilic acid derivatives by reacting p-aminobenzoic acid to HCl salt of ammonium benzoyl chloride. Then condensation of HCl salt of ammonium benzoyl chloride with anthranilic acid and its derivatives at various conditions like temperature, condition of reactants and solvents were maintained for the preparation of dyes. All the dyes were screened for antibacterial activity against *S.aureus* and *E.coli*. These dyes were also shows significant anti-inflammatory activity. The structures of new synthesized dyes were established on the basis of elemental analysis, UV, IR and NMR.

Key words: Anthranilic acid, Thionyl chloride, p-aminobenzoic acid, Pyridine.

INTRODUCTION

Various dyes have been used as therapeutic agents. Ehrlich introduced azo dyes which stain certain tissues[1]. In the year 1932, Domagk introduced 'Prontosil' (4-Sulphonamido-2, 4-diaminobenzene) a dye which is a potent antimicrobial agent[2].

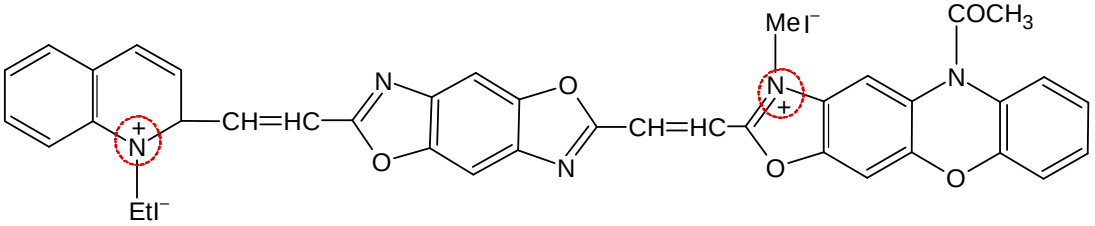
Lesser and Gad, in 1930, patented a method for the preparation of naphtho [1′, 2′, 3′:4-5]-[2, 1-b] quinazoline 5, 10-dione by the reaction of 1-acetamidoanthraquinone with anthranilic acid in the presence of acetic anhydride and zinc chloride. This is found to be useful as a commercial disperse dyestuff for polyester fibre[3].



(I)

Where R_1, R_2, R_3, R_4, R_5, R_6 = H

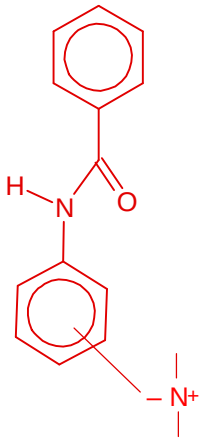
Styryl cyanine dyes find extensive application as photosensitizes for silver halide emulsions, textile dyes and as bactericidal agent[4].



(II)

Anthranilic acid is also well known dye intermediate. If examine structure of dyes reported in literature survey have common –NHCO group which is flanked between two phenyl groups (I) and (II) possesses N+ moiety contributing as Chromophore.

From this assumption we can draw structure of lead molecule for coloration of dye given as below.



The present synthesized dyes also having -COOH group and also structural similarity to p-aminobenzoic acid and sulphonamide and hence contribute in antibacterial activity. Anthranilic acid also has anti-inflammatory activity[5, 6] reported in literature. This basis is taken into consideration for the study of anti-inflammatory activity of synthesized dyes.

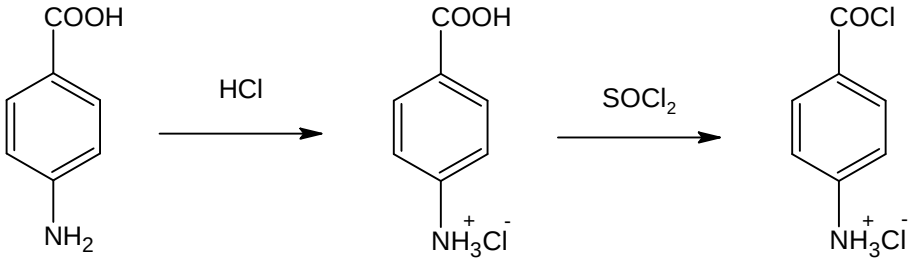
MATERIALS AND METHODS

All the reagents and solvents used were of Laboratory grade. The melting points of dyes were determined by open capillary method and were uncorrected. The IR spectra of dyes were recorded using KBr pellets on FTIR-Vector 22, Bruker, France at Indian Bureau of Mines, Nagpur. The NMR spectra were recorded on Bruker Avance-300 MHz spectrophotometer (Chemical shift in δ ppm) using DMSO as solvent at NIPER, Chandigarh. Antibacterial activity of synthesized dyes was determined by agar plate method[7.] Anti-inflammatory activity of the synthesized dyes was determined by Carrageenan induced rat paw edema method using plethysomometer[8].

EXPERIMENTAL

Step 1:

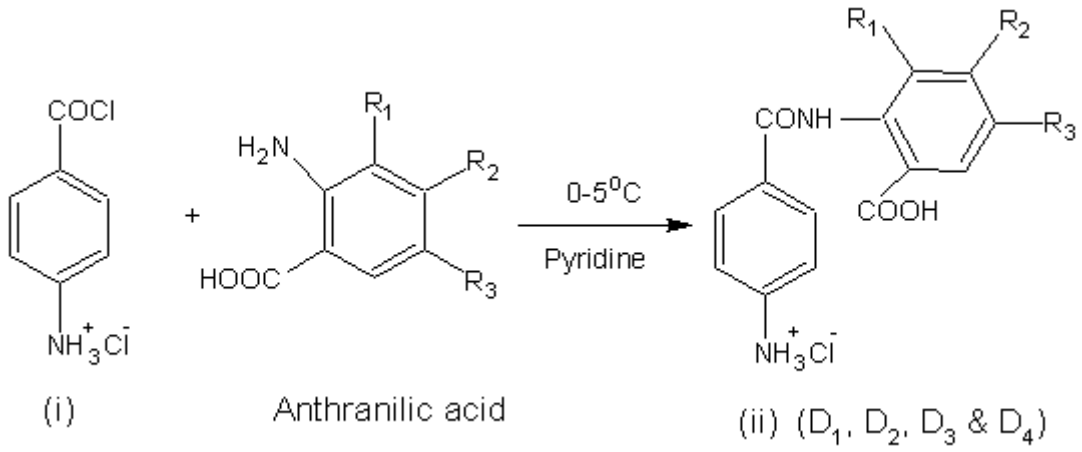
Conversion of p-aminobenzoic acid to hydrochloride salt of ammonium benzoyl chloride (i).



p-aminobenzoic acid (i)

Step 2:

Condensation of (i) with anthranilic acid and anthranilic acid derivatives such as 3, 5-Dibromoanthranilic acid, 5-Bromoanthranilic acid, 5-Iodoanthranilic acid at various conditions like temperature, concentration of reactants and solvents.



Where,

$R_1 = R_2 = R_3 = H$ (D_1)

$R_1 = Br$, $R_2 = H$, $R_3 = Br$ (D_2)

$R_1 = H$, $R_2 = H$, $R_3 = Br$ (D_3)

$R_1 = H$, $R_2 = H$, $R_3 = I$ (D_4)

D_1 = [(4′-ammonium chloride phenyl) amido, N-2 benzoic acid]

D_2 = [(4′-ammonium chloride phenyl) amido, N-2 (3, 5-dibromo) benzoic acid]

D_3 = [(4′-ammonium chloride phenyl) amido, N-2 (5-bromo) benzoic acid]

D_4 = [(4′-ammonium chloride phenyl) amido, N-2 (5-iodo) benzoic acid]

Procedure for the synthesis of dyes

I) D_1: [(4′-ammonium chloride phenyl) amido, N-2 benzoic acid]

The p-aminobenzoic acid (5 g, 0.025mol) was dissolved in dry pyridine (10 ml) and chilled for half an hour to 0 to 5 °C. To it, added thionyl chloride (2 ml, 0.0275mol) drop wise maintaining temperature

below 5 °C. The above mixture poured in solution of anthranilic acid (5 g, 0.0416 mol) and dry pyridine portion wise and allowed to stand for 30 min. at room temperature and diluted it with 0.1N HCl (50 ml).

The separated red dye filtered with suction and dried in atmospheric temperature. The red dye obtained was recrystallized from mixture of n-butyl alcohol and N, N-Dimethylformamide (6:4) and stored in desiccators.

Yield: 6.8 g (85.04%), mp 280-282°C, Rf 0.67 (N,N-dimethylformamide:benzene:water, 7:2:1), λ_{max} 322 nm (N,N-dimethylformamide), IR (KBr, cm^{-1}) 3123-2591 (NH^{+}_{3} stretching), 3161-3024 (Ar-H stretching), 3433-3323 (Ar-NH stretching) 1643 (Ar–COOH stretching) (NMR DMSO-d_6, δ ppm) 2.23 (s, 1H, NH^{+}_{3}), 10.30 (s, 1H, -COOH), 8.4 (s, 1H, NH), 7.78-10.3 (m, 8H, Ar-H).

The same procedure were used to prepare D_2, D_3 and D_4 dyes using 3,5-Dibromoanthranilic acid (5.3 g, 0.0179 mol), 5-Bromoanthranilic acid (5.1 g, 0.0296 mol) and 5-Iodoanthranilic acid (5.5 g, 0.0208 mol) respectively.

II) D_2: [(4′-ammonium chloride phenyl) amido, N-2 (3, 5-dibromo) benzoic acid

Yield: 8.9 g (61%), mp 290-292°C, Rf 0.75 (N, N-dimethylformamide:benzene:water, 7:2:1), λ_{max} 324 nm (N,N-dimethylformamide), IR (KBr, cm^{-1}) 3121-2561 (NH^{+}_{3} stretching), 3221-3196 (Ar-H stretching), 3350 (Ar-NH stretching), 1647 (Ar–COOH stretching), 692-541 (Ar-Br stretching).

III) D_3: [(4′-ammonium chloride phenyl) amido, N-2 (5-bromo) benzoic acid]

Yield: 4.2 g (44.60%), mp 280-282°C, Rf 0.69 (N, N-dimethylformamide:benzene:water, 7:2:1), λ_{max} 324 nm (N, N-dimethylformamide), IR (KBr, cm^{-1}) 3123-2591 (NH^{+}_{3} stretching), 3234-3188 (Ar-H stretching), 3356 (Ar-NH stretching), 1677 (Ar–COOH stretching), 762-537 (Ar-Br stretching).

IV) D_4: [(4′-ammonium chloride phenyl) amido, N-2 (5-iodo) benzoic acid]

Yield: 3.5 g (48.58%), mp 290-294°C (n-butyl alcohol, N, N-dimethyl formamide, 6:4), Rf 0.70 (N, N-dimethylformamide:benzene:water, 7:2:1), λ_{max} 315 nm (N, N-dimethylformamide), IR (KBr, cm^{-1}) 3128-2681 (NH^{+}_{3} stretching), 3234-3188 (Ar-H stretching), 3386 (Ar-NH stretching), 1752 (Ar–COOH stretching), 623-417 (Ar-I stretching).

Antimicrobial activity

The antimicrobial activity of all the new synthesized dyes was determined by cup-plate method in nutrient agar. The antibacterial activity of the dyes was determined against *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram –ve). The solutions of all dyes were made in 70% dimethylformamide in 20% concentration. The petridishes used for antibacterial screening were incubated at 37±1°C for 24 hours; the diameters of zone of inhibition (mm) surrounding each of the wells were recorded. The results were compared with Tetracycline of 100 µg/ml concentration. The results were presented in Table no.1.

Table No.1.Results of Antimicrobial Activity

Dyes	***E. Coli***	***S. Aureus***
	Zone of inhibition (mm)	
Tetracycline	28	29
D_1	25	26
D_2	24	22
D_3	27	25
D_4	28	25

Anti-inflammatory activity

After Carrageenan injection, edema develops in rat paw. The suspension of all the dyes (D_1 to D_4) and Indomethacin, as a standard were prepared using saline and gum acacia as suspending agent. Albino rats were used in a group of five animals. Then, the test preparation was administered orally. The control group was given only suspension of gum acacia. After a 1 hour 0.1ml of Carrageenan was injected into the planar

tissue of right hind paw and immediately paw volume was measured. The same experiment was done using Indomethacin at dose 100 mg/kg body weight. The results were presented in Table no.2.

Table No. 2. Result of Anti-inflammatory Activity

Sr. No.	Group	Oral dose (mg/kg)	Average Paw Volume after Drug Administration			% of Inflammation	
			1h	2h	3h	1h	3h
1.	Control group	50	1.72	1.98	2.46	-	-
2.	Standard	100	1.42	1.39	1.49	29	39
3.	D_1	50	1.63	1.22	1.45	38	41
4.	D_2	50	1.69	1.21	1.32	39	46
5.	D_3	50	1.38	1.34	1.40	32	43
6.	D_4	50	1.46	1.28	1.30	35	47

The activity of the drug is expressed as percent inhibition of edema

$$\text{Percentage of inhibition} = (1-Vt/Vc) \times 100$$

Where, Vt and Vc are the average increase in paw volume of drug treated and control group respectively.

RESULTS AND DISCUSSION

The Dyes ($D_1$to D_4) were prepared from anthranilic acid and its derivatives by various steps. The structures of the dyes were confirmed by spectra and analytical studies. Many antibiotics possesses –COOH group. The present synthesized dyes (D_1 to D_4) also possess –COOH group which is necessary for antibacterial activity and also superimposible on sulphonamides. These dyes have antibacterial property at 20 µg/ml concentration as shown in Table no.1. All above mentioned dyes (D_1 to D_4) were also found to possess satisfactory anti-inflammatory activity shown in Table no.2.

CONCLUSION

The present synthetic scheme for the formation of dyes from anthranilic acid is new. This method is economic, time saving, easy and has structure simplicity. It is observed that these synthesized dyes have broad spectrum antibiotic property.

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Correspondence address:

Ms. P.D. Gaikwad*
Sharad Pawar College of Pharmacy, Wanadongri, Nagpur - 441 110 (M.S.) India
Phone No. 7104-236352
Fax No. 7104-235087
E-mail: *preeti_gaikwad2002@yahoo.com*