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SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME NEW AZO DYES

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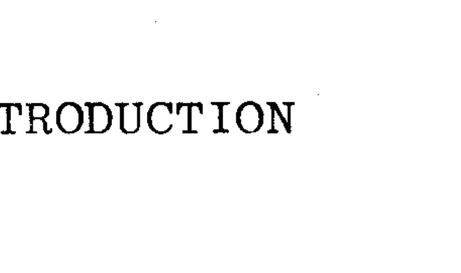
ABSTRACT

The synthesis of some new azo dyes is described. Their structures were confirmed by microanalyses, IR, NMR and MS. The anti-inflammatory and antimicrobial activities of some of them were determined.

INTRODUCTION

Ulcerative colitis is a disease of unknown oetiology characterised by inflammation. In the early 1940's Svarts developed a particular azo dye, Salicylazosulphapyridine (SASP) of unique effectiveness against ulcerative colitis[1,2]. Accordingly , we decided to prepare certain structurally related azo dyes having the structure (1) to be screened as antiinflammatory or antimicrobial agents.



(1)

R=Different Sulphonamides, R'=NH_2, R'' = OH, SH, NH-C_2H_5, N-p-tolyl, m-trifluoromethylphenylamino, 2,3-dimetnylphenylamino.

The choice of thiosalicylic and anthranilic acids and their derivatives is based on isosteric replacement of OH group with SH or NH_2 and the fact that some anthranilic acid derivatives are used as antiinflammatory agents with the hope of preparing less toxic and more potent drugs.

The choice of PAS is based on the fact that it is structurally related to 5-aminosalicylic acid which has proved to be effective against ulcerative colitis. The newly synthesized drugs may act as prodrugs affording 5-aminosalicylic acid in the colon since SASP itself is considered as a prodrug.

EXPERIMENTAL

IR KBr, spectra were recorded on a Pye-Unicam SP-1000 spectrophotomcter. For NMR spectra, a Geol 90 MHZ spectrometer was used. Gas-Mass spectra were obtained by AE 20 spectrometer with computer printer-plotter. Micronalyses were carried out at the microanalytical unit, Cairo University

Reported procedures were adopted to prepare thiosalicylic[3] and the folloiwing acids: N-P-tolylanthranilic[4], mephenamic[5], fluphenamic[6], P-Aminosalicylic[7], and N-ethylanthranilic[8].

Azo Sulphomides, General Procedure:

The azo dyes (Tables 1-5) were prepared by coupling the diazotized appropriate sulphonamide with the appropriate acids.

Diazotization:

a- The sulphonamide (0.01 mole) was dissolved in conc HCl 20 ml, diluted to 100 ml with water and cooled to $5^{o}C$. Cold solution of sodium nitrite in water (0.01 mole) was added. The solution was set aside for 15 min after the addition of sodium nitrite solution at a temperature not exceeding $5^{o}C$.

b- The sulphonamide (0.01 mole) was dissolved in Sodium Hydroxide 25% (20 ml). Sodium nitrite (0.012 mole) was added with contineous stirring and acidified by the dropwise addition of HCl (25 ml).

Coupling Procedure

The diazonium salt solution prepared as above was gradually added with contineous stirring to a solution of salicylic acid (0.01 mole), thiosalicylic acid, N-ethylanthranilic, mephenamic, fluphenamic N-p-tolylanthranilic acid .. PAS (0.01 mole of each), 1.2 gm of potassium hydroxide and 0.6 gm of sodium carbonate. The medium was maintained just alkaline as necessary . In all cases, the azo dyes were isolated, adjusting the pH of the mixture to 5-6 by the addition of dilute HCl (10%) solution with stirring. In all cases the dyes were purified by dissolving in sodium hydroxide solution followed by precipitation with dilute HCl followed by recrystallization from DMF and water.

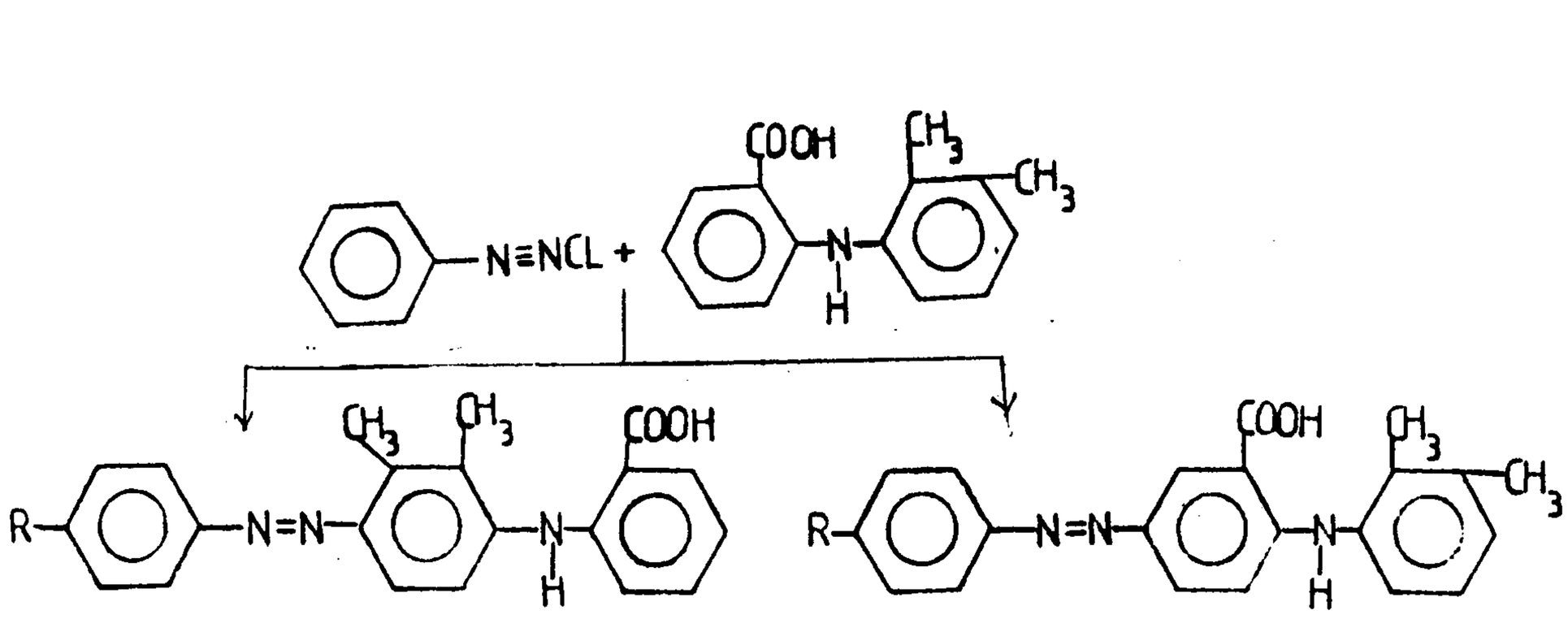
RESULTS AND DISCUSSION

SASP and the new azo dyes were prepared by diazotizing the appropriate sulphonamides and coupling the diazonium salt with salicylic, thiosalicylic, anthranilic, mephenamic, fluphenamic, N-ethyl and N-p-tolylanthranilic acids in alkaline medium. In spite of the fact that the method of preparation of the new dyes looks simple, the nature and the number of impurities associated with the synthesis of SASP and the proposed new compounds are of considerable importance and interest to regulatory agencies, the USP-NF[9] and the manufactures of drug subatances because some of these impurities are biologically inactive and toxic.

Salipsky et al[10] isolated the following compounds from SASP and characterised them by mass spectrometry : (pyridylsulphamoylphenylazophenyl), 3-(p-2-pyridylsulphamoylphenylazo)-salicylic acid, unreacted sulphapyridine, 5-(2-pyidylphenylaminophenylazo)-salicylic acid in addition to a benzyne polymer.

Accordingly, purification of the new dyes was accomplished by several recrystallizations followed by TLC. The structures and purity of the new azo dyes were confirmed by microanalysis, IR, NMR and GM-spectra

In the case of mephenamic acid, there are mainly two possibilities for coupling, one in the benzene ring of anthranilic acid and the other in the benzene ring of o-xylene



Coupling occurs mainly in the benzene ring of anthranilic acid due to NH_2 group (ortho-para director) and carboxyl group (meta director) and the steric hindrance in the benzene ring of o-xylene . This was proved by NMR and mass spectrometry. In the NMR spectra, the upper field region

showed two distinct signals at (δ 2.1 and 2.3) p.p.m (3H each) assignable to the two methyl groups in the mephenamic acid moiety.

A further singlet appeard in the spectrum of mephenamazosulphacetamide at (δ2.0) p.p.m., (3H), which is ascribed to the acetyl group protons in the respective molecule. The spectra showed multiplet centered at (δ 6.5-6.9) p.p.m.,assignable to the four protons of the phenyl group of the sulpha moiety, another multiplet centered at (δ 7-7.5) due to the three protons of anthranilic acid moiety and the three protons of the phenyl group of the o-xylyl moiety appeared as a multiplet at (7.8-8.5) p.p.m. The absence of the following pattern;(doublet,triplet, triplet, doublet) which is assigned to the four adjacent protons of anthranilic acid moiety, confirms the coupling in the benzene ring of anthranilic acid.

Further confirmation of the structure was obtained from the IR spectra showing no band at 750 cm^{-1} indicating the absence of 4 adjacent CHs.

For the mass spectra,most of the prepared compounds were unstable since no measurable molecular ions were detected except in the case of mephenazosulphacetamide.

The same reasoning can be used to prove the structures of the fluphenamazosulphonamides (Table4) in addition to the fact that the trifluoromethyl group will deactivate the benzene ring towards coupling with the diazonium salt of the sulphonamides.

Biological Testing

Antiinflammatory activities:

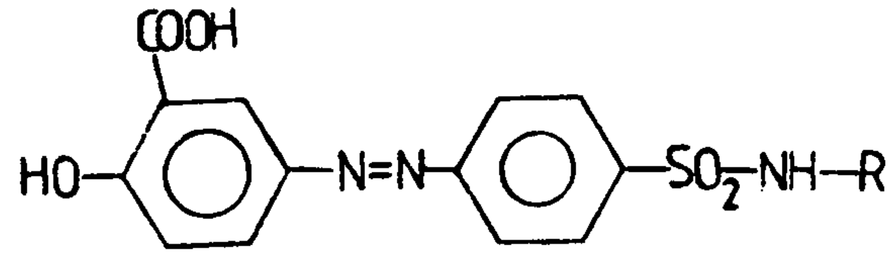
Antiinflammatory activity of some representative examples of the new dyes was studied using the carragineen-induced oedema. Salazopyrine (Pharmacia , Sweden) was used as reference. The test drug and reference were injected i.p. in a dose of 10 mg/kg one hour before carragineen injection. A group of 6 rats receiving carragineen alone served as a control (Table 6 Fig. 1). All tested compounds are found active against carragineen-induced oedema . The percentage reduction of oedema ranged from 62-69% of the control. All tested compounds are nearly of the same potency and nearly as potent as the reference standard SASP.

Antimicrobial Testing:

The antimicrobial testing showed that most of the new compounds are very active against Staph aureous, B.subtilis, P.mirabilis. None of them are active aginst K.pneumonia or Candida albicans. The most interesting is the variable sensitivity of Compounds 1 and 7 against P.aerogenosa (Table 7).

Salicylazosulphonamides

Table No. (1)



No	R	Solvent of crystallization	m.p.°C (dec)	% Yield	Formula	Analysis	
						Calcd.	Found
I	2-pyrimidinyl	DMF/ Water 1:2	220-226	90	$C_{17}H_{13}N_5O_5S$	C 51 H 3.5 S 8.0	50.5 4.7 7.8
II	2-thiazolyl	DMF/ water 1:1	231-235	65	$C_{16}H_{12}N_4O_5S_2$	N 13.8	14.0
III	Acetyl	DMF/ water 1:1	222-224	88	$C_{15}H_{13}N_3S$	S 8.8	9.[illegible]
IV	2-Pyridyl	DMF/ Water 1 : 2	225-228	90	$C_{14}H_{14}N_5O_5S$	C 46.1 H 3.8 S 8.7	45.8 4.0 8.7

Anthranilazosulphonamides

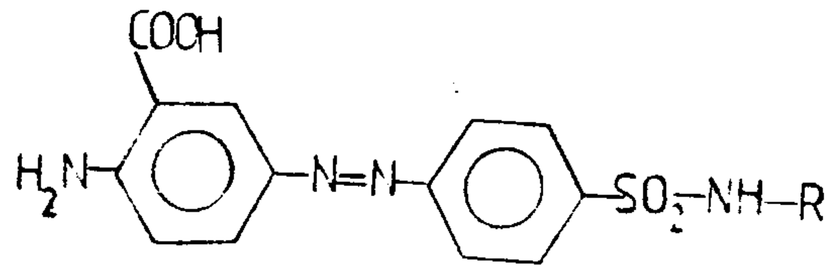


Table No.(2)

No	R	Solvent of crystallization	m.p.°C (dec)	% Yield	Formula	Analysis	
						Calcd.	Found
I	2-pyrimidinyl	DMF/ H_2O 1 : 1	237-239	92	$C_{17}H_{14}N_6O_4S$	N 21.1 S 8.0	20.8 8.2
II	2-thiazolyl	DMF/H_2O 1:2	238-240	60	$C_{16}H_{13}N_5O_4S_2$	N 17.3 S 15	17.5 15.9
III	Acetyl	DMF/ H_2O 1:2	225-228	82	$C_{15}H_{14}N_4O_5S$	S 8.8	8.9
IV	2-Pyridyl	DMF/ H_2O 1:1 1:2	224-226	83	$C_{18}H_{15}N_5O_4S$	S 8.1	8.6

I.R.Spectra: cm^{-1}
3345 cm^{-1} NH stretch, 1672 : carbonylstretch,
2500-3200 :broad absorption (COOH).

p-(2-pyridylsulphamoyl-phenylazo)-N-p-tolyl anthranilic acid:
m.p. 267-271 (dec)
Yield: 71%
Analysis: S Calcd (6.5) Found (6.8)
p-(2-pyrimidinyl-sulphamoyl-phenylazo)-N-ethyl anthranilic acid:
m.p.: 266-268 (dec)
Yield: 73%
Analysis: S:Calcd (7.5), Found (7.9)

Mephenamazosulphonamides

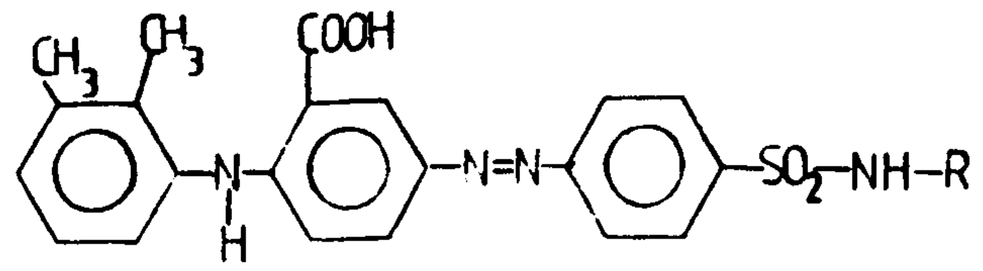


Table No.(3)

No	R	Solvent of crystallization	m.p.°C (dec)	% Yield	Formula	Analysis	
						Calcd.	Found
I	2-pyrimidinyl	DMF/ H_2O 1:1	245-248	90	$C_{25}H_{22}N_6O_4S$	C 59.7 H 4.3 S 6.3	60 4.3 5.8
II	2-thiazolyl	DMF/ H_2O 1:2	256-259	60	$C_{24}H_{21}N_5O_4S_2$	N 17.3 S 15.6	17.5 15.9
III	Acetyl	DMF/ H_2O 1:2	240-242	89	$C_{23}H_{22}N_4O_5S$	N 12 S 6.8	11.6 6.5
IV	Quanidin	DMF/H_2O 1:2	230-237	89	$C_{22}H_{22}N_6O_4S$	S 6.8	6.9
V	Pyridyl	DMF/H_2O 1:2	235-238	77	$C_{26}H_{22}N_5O_4S$	S 6.4	6.4
VI	4,6-dimethyl-2-Pyrimidyl	DMF/H_2O 1:1	246-250	72	$C_{27}H_{26}N_6O_4S$	S 6.6	7.0

Flufenamazosulphonemides

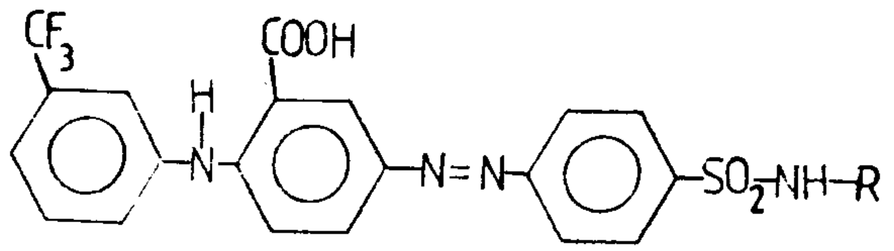


Table No.(4)

No	R	Solvent of crystallization	m.p.°C (dec)	% Yield	Formula	Analysis	
						Calcd.	Found
I	2-pyrimidinyl	DMF/ H_2O 1:1	270-277	78	$C_{24}H_{17}N_6S\,O_4F_3$	N 15.4 S 5.5	15.3 5.6
II	2-thiazolyl	DMF/ H_2O 1:2	276-280	60	$C_{23}H_{16}N_5S_2O_4F_3$	S 11.7 F 10.4	12.2 10.9
III	Acetyl	DMF/ H_2O 1:1	250-256	70	$C_{22}H_{17}N_4S\,O_5F_1$	N 11.0	11.3
IV	Guanidin	DMF/ H_2O 1:1	241-244	72	$C_{21}H_{17}N_6S\,O_4F_3$	S 6.3	6.3
V	2-Pyridyl	DMF/H_2O 1:2	242-244	79	$C_{25}H_{18}N_5O_4S\,F_3$	C 55.4 H 3.5 N 12.9 S 5.9	55.3 3.5 13.2 6.4
VI	4,6 dimethyl-2-Pyrimidyl	DMF/ H_2O 1:2	261-269	75	$C_{26}H_{21}O_4N_6S\,F_3$	S 5.6	5.6

I.R.: Spectra cm^{-1}
1600 cm^{-1} : sromatic atretch.
2500-3200: broad absorbation (COOH).
1670 :Carbonyl stretching.

Paraminosalicylazosulphonamides

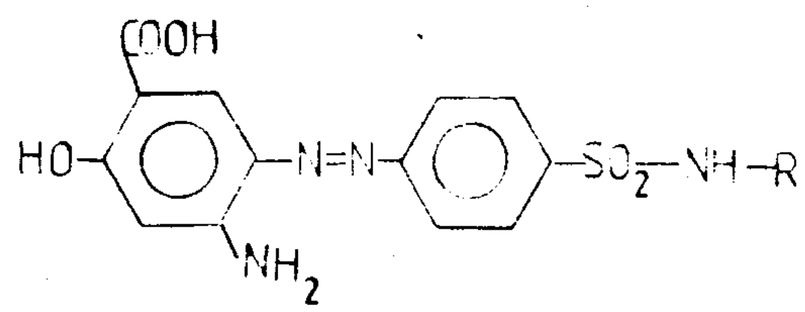


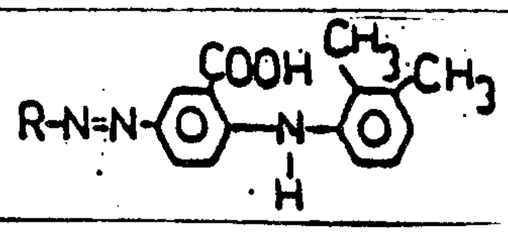
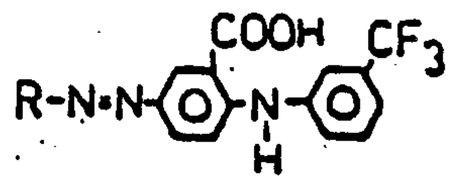
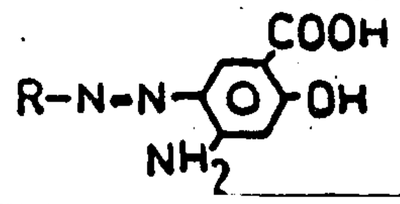
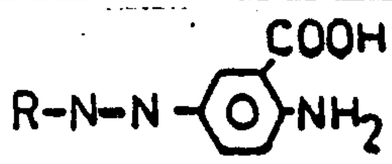
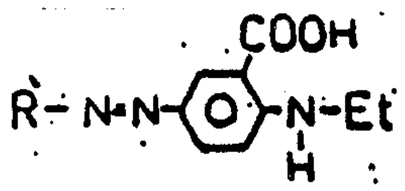
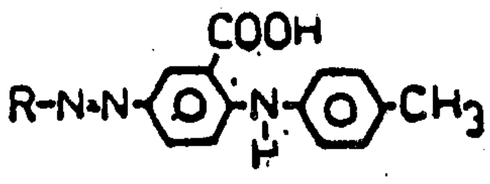
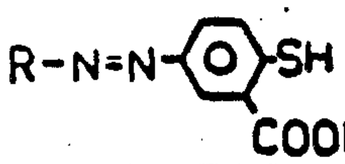
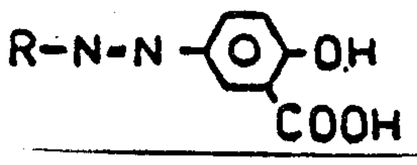
Table (5)

No	R	Solvent of crystallization	m.p.°C (dec)	% Yield	Formula	Analysis	
						Calcd.	Found
I	2-pyrimidinyl	DMF/ H_2O 1:1	231-233	79	$C_{17}H_{14}N_6O_5S$	N 20.2 S 7.7	20 7.7
II	2-thiazolyl	DMF/ H_2O 1:2	238-240	62	$C_{16}H_{13}N_5O_5S_2$	N 16.7 S 15.2	16.5 15.2
III	Acetyl	DMF/ H_2O 1:1	224-226	78	$C_{15}H_{14}O_6N_4S$	N 14.8 S 8.4	14.6 8.5
IV	Guanidin	DMF/ H_2O 1:2	225-228	72	$C_{14}H_{15}N_6O_5S$	N 22.2 S 8.4	22 8.4
V	2-Pyridyl	DMF/H_2O 1:1	230-231	81	$C_{18}H_{15}N_5O_5S$	N 16.9 S 7.7	16.8 7.8
VI	4,6 dimethyl-2-Pyrimidyl	DMF/ H_2O	236-239	74	$C_{19}H_{18}O_5N_6S$	N 19.0 S 7.2	18.7 7.3

I.R. Spectra: Cm^{-1} : 850 : C-N ; 1600: -N=N- ; 1672 : of carbonyl group

Table (6)

Effect of Salazopyrine and test compounds on carrageenin-induced oedema of the rat paw

Compound No.	Chemical structure	Dose (mg/kg)	Weight of the rat paw (mg) $\bar{x}$ + S.E	Percentage change of control value
Control carrageenin (0.05ml/rat)	-	-	290 ± 2.88	100.00
1		10	100^{X} ± 1.18	34. 8
2		10	110.8^{X} ± 3.25	38.21
3		10	100^{X} ± 3.65	34.48
4		10	89.5^{X} ± 3.54	30.86
5		10	103.5^{X} ± 1.22	35.86
6		10	102^{X} ± 4.49	35.17
7		10	109.1^{X} ± 8.79	37.62
Salazopyrine (Standard)		10	107.8^{X} ± 2.08	37.17

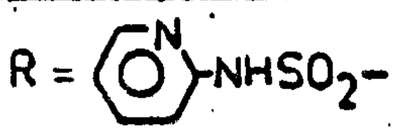
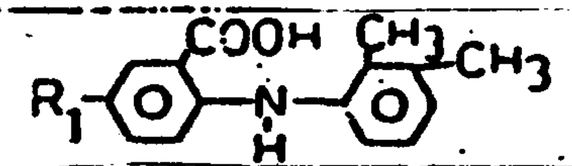
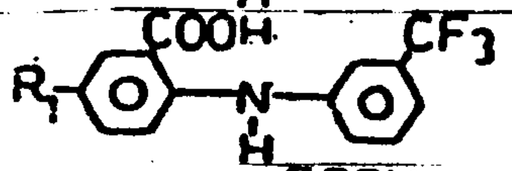
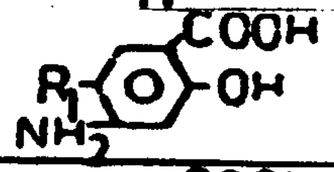
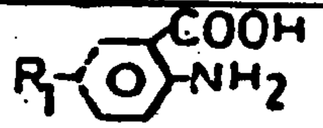
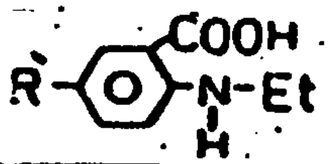
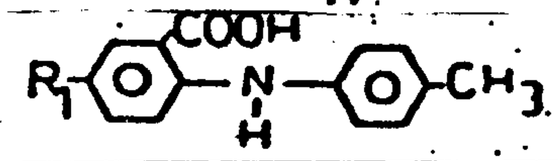
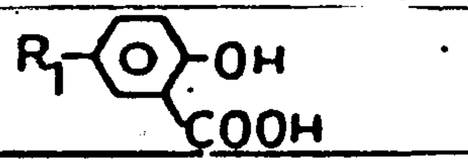
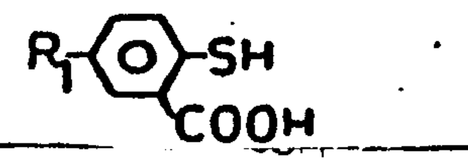
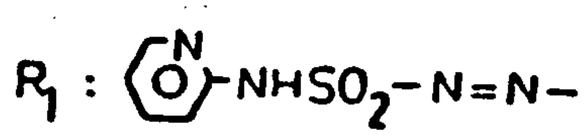
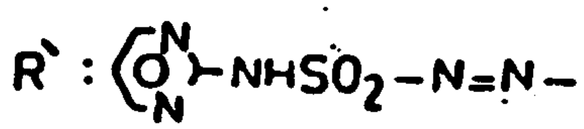
R = 

Table (7) ANTIMICROBIAL TESTING OF THE NEW AZO DYES

Microorganisms / Structure	Staphylococcus aureus	Bacilles subtilis	Pseudomonas aeruginosa	Proteus virabilis	Escherichia coli	Klebsiella pneumonia	Candida albicans
	14	R	V	16	R	R	R
	11	12	R	R	R	R	R
	10	R	R	11	R	R	R
	R	R	R	R	R	R	R
	R	R	R	10	R	R	R
	11	R	R	R	R	R	R
	10	R	R	R	R	R	R
	15	16	V	16	R	R	R

Diameter of the inhibition zone by mm.
Concentration of the tested compound, 1 mg/ml
Solvent dimethyl formamide.
R = Resistant.
V = Variable.

R_1 : 

R' : 

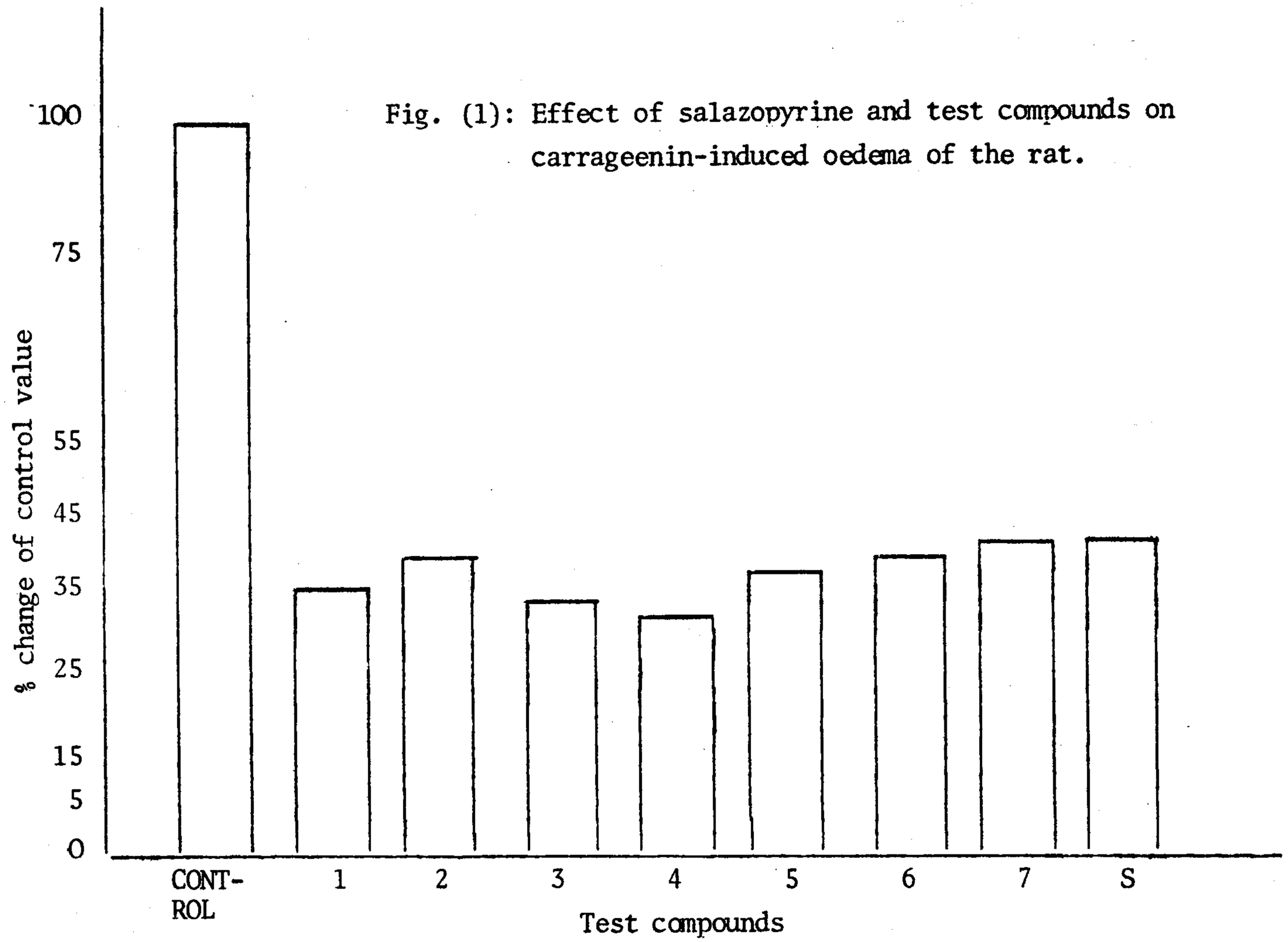


Fig. (1): Effect of salazopyrine and test compounds on carrageenin-induced oedema of the rat.

S = Reference standard salazopyrine

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تخليق بعض المركبات الصبغية الجديدة بهدف اختبارها اقربازينيا

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امكن تخليق بعض الصبغات الجديدة بتفاعل الملح الديازونيومى لبعض مركبات السلفا مع حامض الساليسليك الثيوساليسليك والانثرانيليك والمفيناميك والفلوفيناميك .

وتم التعرف على صيغتها البنائية باستعمال مطياف الكتلة والرنين النووى المغناطيسى والاشعة دون الحمراء والتحليل الدقيق وثبت ان بعض هذه المركبات لها فاعلية ضد الالتهابات وضد بعض الميكروبات .

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