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Synthesis and Antimicrobial Activity of Novel Formazans and Their Tetrazolium Bromides

R.T. VASHI* and N.M. SHETH
Department of Chemistry, Navyug Science College, Surat-395 009, India
E-mail: vashirajendra@yahoo.co.in; nmsheth13@yahoo.co.in

Novel formazans [**III(a-l)**] have been synthesized by the coupling reaction of diazotized solution of substituted sulfanilamide derivatives with aryl hydrazones of different benzaldehydes in pyridine. The resultant formazans were further cyclized by H_2SO_4,H_2O_2/Fe^{2+}, NaBr to prepare the corresponding tetrazolium bromides [**IV(a-l)**]. The compounds were characterized by ^{1}H NMR, IR and elemental analysis. The compounds were assayed for antimicrobial activity.

Key Words: Novel formazans, Tetrazolium bromides, Antimicrobial activity.

INTRODUCTION

Sulfanilamide as sulfa drug and biological activities of formazans are well known. Tetrazolium salts are used as staining and visualization reagent in neoplastic tissues. The formazans and tetrazolium salts synthesized from the indole, thiadiazole, quinoline, quinazoline, benzimidazole derivatives are known for their biological activities[1-6]. Formazans have been found to possess important medical applications. The tetrazolium salts are classified as promoter of vitality formazans and heterocyclic hyrazones are known for their spectrum of biological activities such as antiviral[7], antimicrobial[8], anticancer[9] and antifungal[10]. The synthesis of some formazans from sulfa drugs and their tetrazolium bromides have also been reported[11]. Recently thiazolyl/oxazolyl indole formazans were tested for antiinflammatory activity[12] and aryl formazans were tested for their antimicrobial activities[13]. These findings concerning importance of sulfanilamide moiety in biological system and biological activities of formazans, prompted us to synthesize formazans and its tetrazolium salt using sulfanilamide as synthons.

EXPERIMENTAL

The sulfanilamide derivatives were prepared according to the general method reported in literature[14]. The aryl hydrazones of substituted benzaldehyde were prepared according to reported method for synthesis of hydrazones[15]. All chemicals used for synthesis were of extra pure grade.

Measurement of infrared (IR) spectra were taken on a Perkin-Elmer-983, spectrophotometer with KBr pellets, NMR were recorded on Bruker AC 300/400 MHz FT-

NMR in DMSO-d_6, melting points were taken in open capillaries and are uncorrected. Purity of the compounds was checked on gilica gel G, Merk 254 plates using chloroform and methanol as the solvent system (9:1) and the bromide salts were confirmed by the halogen test. The compounds have been assigned the structures on the basis of their elemental analyses and spectral data.

Preparation of formazans [III(a-l)]: To a stir solution of [**II(a-l)**] (1.72 g, 0.01 mol) in mixture of acetic acid and conc. hydrochloric acid (50:50 v/v, 20 mL), an aqueous solution of sodium nitrite 10 mL (0.79 g, 0.01 mol) in water was added by maintaining the temperature in the range of 0-5 ºC. The diazotized **II(a-l)** was gradually added to stir solution of phenyl hydrazone derivative [**I(a)**] in pyridine (20 mL) below 10 ºC. The reaction mixture was allowed to attain RT and stand overnight at room temperature. The reaction mixture was quenched by pouring in crushed ice. The precipitated solid product was washed with petroleum ether (40-60 ºC) and then purified by recrystallization in methanol to furnish pure formazan. The percentage yield were in the range of 40-65 %. Analytical results shown in Tables 1-3.

Preparation of tetrazolium salt [IV(a-l)]: A solution of formazan (**IIIa**, 0.01 mol) in methanol/dioxane was added to a 2 N H_2SO_4 (5 mL) containing traces of ferrous sulphate. 30 % hydrogen peroxide solution (2 mL) was added and the reaction mixture was heated on water bath for 5 h. The completion of oxidation was indicated by disappearance of colour.

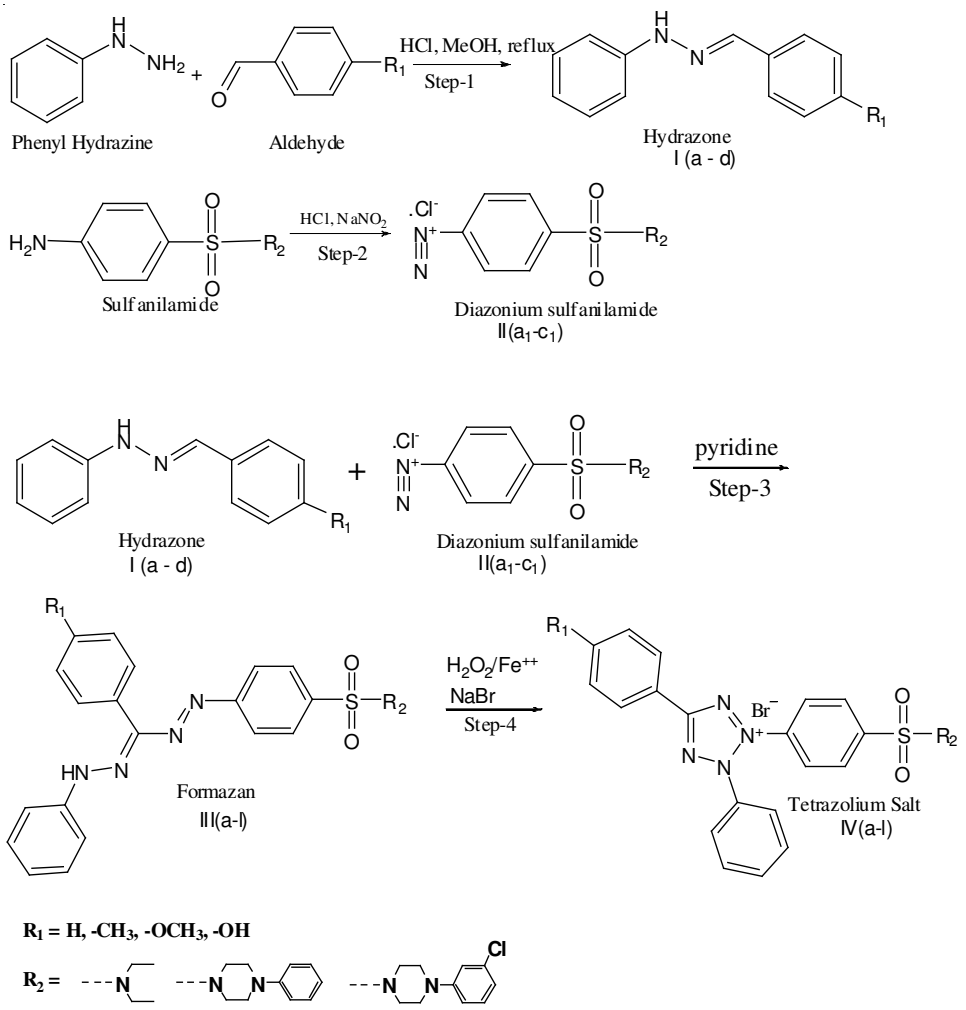
Excess methanol/dioxane was distilled off and obtained syrup was treated with excess sodium bromide to precipitate out the crude tetrazolium bromide. The solid product was filtered, washed with a little water, dry it in air and then triturated with petroleum ether (60-80 ºC), to furnish tetrazolium bromide with percentage yield in the range of 35-50 %. The isolated product was stored in refrigerator at 4 ºC. Analytical results are shown in Tables 1-3.

The reaction scheme for synthesis of formazans and tetrazolium salts have been presented in **Scheme-I**.

Antimicrobial activity: The formazans and their tetrazolium salts were studied for antimicrobial activity against gram positive (*B. subtillis* and *S. aureus*) and gram negative (*E. coli* and *P. aeruginosa*) organisms using DMF as solvent at 50 mg/mL concentration at 37 ºC. The zone of inhibition was measured in mm. The activity was compared with the known antibiotics *viz*., ampicillin, tetracycline, gentamicin and chloramphenicol at same concentration by cup-plate method[16], which is reported in the Table-1.

RESULTS AND DISCUSSION

The novel formazan derivates **III(a-l)** are formed by reaction between hydrazones of aldehyde and diazonium chloride of sulfanilamide. Hence the IR spectra of all **III(a-l)** compounds comprise the frequencies of both known reagent. Thus the bands due to aromatic rings, azo group, hydrazones groups are at their



Scheme-I

respective positions. There is no any appreciable change in the spectra of formazans **III(a-l)** compared to those of **I** and **II**. However IR observation of **IV(a-l)** tetrazolium salts of formazans **III(a-l)**, reveal that the disappearance of the frequency at 3200 cm^{-1} due to -NH group. Hence it confirms the salt formation. The NMR spectra of soluble tetrazolium salts also indicate that the absence of signal due to -NH group.

Antimicrobial activity: The formazans and their tetrazolium salts were assayed for antimicrobial activity against gram positive and gram negative bacteria. The zone of inhibition was measured in mm. The activity was compared with the standard drugs *viz.*, ampicillin, tetracycline, gentamycin and chloramphenicol at same concentration (Table-1). The compounds **IIIb**, **IIIc**, **IIIe**, **IIIf**, **IIIh**, **IIIi**, **IIIk**, **IVb**, **IVe**,

TABLE-1
PHYSICAL DATA AND ANTIMICROBIAL ACTIVITY OF FORMAZANS AND ITS TETRAZOLIUM SALTS

Compound	R_1	R_2	m.p. (°C)	N Calcd. (found.) (%)	Gram +Ve		Gram –Ve	
					B. Subtillis	*S. Aureus*	*E. coli*	*P. Aeruginosa*
IIIa	H	$N(C_2H_5)_2$	151	16.08 (16.00)	09	07	08	06
IIIb	H	$N(C_2H_4)_2NC_6H_5$	161	16.02 (15.90)	17	16	15	12
IIIc	H	$N(C_2H_4)_2NC_6H_4Cl$	164	15.03 (14.90)	18	19	16	14
IIId	CH_3	$N(C_2H_5)_2$	182	15.58 (15.50)	11	11	09	10
IIIe	CH_3	$N(C_2H_4)_2NC_6H_5$	178	15.60 (15.50)	18	17	14	13
IIIf	CH_3	$N(C_2H_4)_2NC_6H_4Cl$	183	14.66 (14.60)	19	20	17	16
IIIg	OCH_3	$N(C_2H_5)_2$	180	15.04 (14.90)	10	11	10	10
IIIh	OCH_3	$N(C_2H_4)_2NC_6H_5$	173	15.15 (15.00)	19	18	15	14
IIIi	OCH_3	$N(C_2H_4)_2NC_6H_4Cl$	179	14.27 (14.10)	23	23	16	15
IIIj	OH	$N(C_2H_5)_2$	194	15.51 (15.40)	09	10	10	10
IIIk	OH	$N(C_2H_4)_2NC_6H_5$	181	15.54 (15.40)	18	17	13	12
III$_l$	OH	$N(C_2H_4)_2NC_6H_4Cl$	177	14.61 (14.60)	20	19	16	17
IVa	H	$N(C_2H_5)_2$	247	13.61 (13.50)	10	11	09	10
IVb	H	$N(C_2H_4)_2NC_6H_5$	232	13.92 (13.80)	15	16	14	16
IVc	H	$N(C_2H_4)_2NC_6H_4Cl$	239	13.17 (13.00)	16	17	16	14
IVd	CH_3	$N(C_2H_5)_2$	211	13.25 (13.10)	11	08	10	11
IVe	CH_3	$N(C_2H_4)_2NC_6H_5$	215	13.01 (12.90)	16	17	15	16
IVf	CH_3	$N(C_2H_4)_2NC_6H_4Cl$	221	12.89 (12.80)	19	18	16	17
IVg	OCH_3	$N(C_2H_5)_2$	219	12.86 (12.70)	10	09	09	11
IVh	OCH_3	$N(C_2H_4)_2NC_6H_5$	216	13.26 (13.10)	16	14	14	15
IVi	OCH_3	$N(C_2H_4)_2NC_6H_4Cl$	238	12.58 (12.50)	18	17	16	17
IVj	OH	$N(C_2H_5)_2$	208	13.20 (13.10)	10	10	10	09
IVk	OH	$N(C_2H_4)_2NC_6H_5$	218	13.56 (13.40)	15	16	15	16
IV$_l$	OH	$N(C_2H_4)_2NC_6H_4Cl$	221	12.85 (12.70)	19	18	17	14
Ampicillin	–	–	–	–	19	15	20	21
Tetracycline	–	–	–	–	21	19	15	24
Gentamycin	–	–	–	–	20	18	19	22
Chloramphenicol	–	–	–	–	20	25	18	24

Give the characteristic halogen test and m.p. as decomposition **[IV(a-l)]**. For antimicrobial activity the zone of inhibition was measured in mm.

IVf, **IVh**, **IVi**, **IVk** and **IVl** exhibited good activity against gram positive (*B. subtillis* and *S. aureus*) and gram negative (*E. coli* and *P. aeruginosa*) bacteria, whereas compounds **IIIa** , **IIId**, **IIIg**, **IIIj**, **IVa**, **IVd**, **IVg** and **IVj** showed moderate to mild activity against all four bacteria as compared to standard drugs.

TABLE-2
^{1}H NMR SPECTRA OF COMPOUNDS

Comp.	Solvent	^{1}H NMR spectrum, δ (ppm)
IIIa	DMSO-d_6	1.15 (t, 6H, CH_3), 3.35 (q, 4H, CH_2), 6.75 (m, 1H, ArH), 7.15 (t, 2H, ArH), 7.4 (dd, 2H, ArH), 7.50-7.53 (m, 3H, ArH), 7.80-7.85 (m, 6H, ArH), 10.2 (brs, 1H, -NH)
IIIb	DMSO-d_6	3.15 (s, 8H, CH_2), 6.75-6.78 (m, 2H, ArH), 6.95 (dd, 2H, ArH), 7.1-7.26 (m, 4H, ArH), 7.4 (dd, 2H, ArH), 7.50-7.53 (m, 3H, ArH), 7.81-7.85 (m, 6H, ArH), 10.3 (brs, 1H, -NH)
IIIc	DMSO-d_6	3.15 (s, 8H, CH_2), 6.80-6.84 (m, 3H, ArH), 6.93 (s, 1H, ArH), 7.20-7.22 (m, 3H, ArH), 7.4 (dd, 2H, ArH), 7.50-7.53 (m, 3H, ArH), 7.81-7.85 (m, 6H, ArH), 10.4 (brs, 1H, -NH)
IIId	DMSO-d_6	1.1 (t, 6H, CH_3), 2.3 (s, 3H, CH_3), 3.30 (q, 4H, CH_2), 6.82 (m, 1H, ArH), 7.22-7.35 (m, 6H, ArH), 7.73 (d, 2H, ArH), 7.85-7.9 (m, 4H, ArH), 10.1 (brs, 1H, -NH)
IIIe	DMSO-d_6	2.32 (s, 3H, -CH_3), 3.17 (s, 8H, CH_2), 6.79-6.81 (m, 2H, ArH), 6.95 (dd, 2H, ArH), 7.25-7.35 (m, 8H, ArH), 7.73 (d, 2H, ArH), 7.85-7.89 (m, 4H, ArH), 10.9 (brs, 1H, -NH)
IIIf	DMSO-d_6	2.32 (s, 3H, -CH_3), 3.17 (s, 8H, CH_2), 6.81-6.84 (m, 3H, ArH), 6.93 (s, 1H, ArH), 7.23-7.35 (m, 7H, ArH), 7.73 (d, 2H, ArH), 7.83-7.86 (m, 4H, ArH), 11.2 (brs, 1H, -NH)
IIIg	DMSO-d_6	1.01 (t, 6H, CH_3), 3.26 (q, 4H, CH_2), 3.80 (s, 3H, OCH_3), 6.82 (m, 1H, ArH), 7.04 (d, 2H, ArH), 7.24 (t, 2H, ArH), 7.34 (dd, 2H, ArH) 7.73 (d, 2H, ArH), 7.83-7.85 (m, 4H, ArH), 11.1 (brs, 1H, -NH)
IIIh	DMSO-d_6	3.2 (s, 8H, CH_2), 3.80 (s, 3H, OCH_3), 6.79-6.82 (m, 2H, ArH), 6.95 (dd, 2H, ArH), 7.06 (d, 2H, ArH), 7.25-7.35 (m, 6H, ArH), 7.72 (d, 2H, ArH), 7.84-7.87 (m, 4H, ArH), 11.0 (brs, 1H, -NH)
IIIi	DMSO-d_6	3.2 (s, 8H, CH_2), 3.83 (s, 3H, OCH_3)), 6.81-6.84 (m, 3H, ArH), 6.93 (s, 1H, ArH), 7.06 (d, 2H, ArH), 7.2-7.23 (m, 3H, ArH), 7.34 (dd, 2H, ArH), 7.73 (d, 2H, ArH), 7.83-7.85 (m, 4H, ArH), 11.13 (brs, 1H, -NH)
IIIj	DMSO-d_6	1.01 (t, 6H, CH_3), 3.29 (q, 4H, CH_2), 6.82-6.85 (m, 3H, ArH), 7.22 (t, 2H, ArH), 7.34 (dd, 2H, ArH), 7.65 (d, 2H, ArH), 7.82-7.84 (m, 4H, ArH), 9.5 (brs, 1H, -OH) 11.1 (brs, 1H, -NH)
IIIk	DMSO-d_6	3.2 (s, 8H, CH_2), 6.82-6.85 (m, 4H, ArH), 6.95 (dd, 2H, ArH), 7.21-7.35 (m, 6H, ArH), 7.66 (d, 2H, ArH), 7.82-7.84 (m, 4H, ArH), 9.1 (brs, 1H, -OH), 11.2 (brs, 1H, -NH)
IIIl	DMSO-d_6	3.2 (s, 8H, CH_2), 6.82-6.85 (m, 5H, ArH), 6.93 (s, 1H, ArH), 7.21-7.23 (m, 3H, ArH), 7.34 (dd, 2H, ArH), 7.66 (d, 2H, ArH), 7.82-7.84 (m, 4H, ArH), 9.1 (brs, 1H, -OH), 10.9 (brs, 1H, -NH)
IVa	DMSO-d_6 and D_2O	1.05 (t, 6H, CH_3), 3.3 (q, 4H, CH_2), 7.39-7.58 (m, 6H, ArH), 7.87-7.91 (m, 4H, ArH), 8.02 (dd, 2H, ArH), 8.1 (d, 2H, ArH)
IVo	DMSO-d_6 and D_2O	3.21 (s, 8H, CH_2), 3.83 (s, 3H, OCH_3), 6.81 (m, 1H, ArH), 6.92 (dd, 2H, ArH), 7.05 (d, 2H, ArH), 7.25 (t, 2H, ArH), 7.45 (m, 1H, ArH), 7.61 (t, 2H, ArH), 7.87-7.91 (m, 6H, ArH), 8.02 (dd, 2H, ArH)
IV_l	DMSO-d_6 and D_2O	3.21 (s, 8H, CH_2), 6.82-6.84 (m, 4H, ArH), 6.94 (s, 1H, ArH), 7.21 (t, 1H, ArH), 7.45 (m, 1H, ArH), 7.61 (t, 2H, ArH), 7.87-7.91 (m, 6H, ArH), 8.02 (dd, 2H, ArH)

TABLE-3
IR SPECTRA OF COMPOUNDS **[III(a-1)]**

Comp.	IR spectrum (KBr) ν in cm^{-1}
IIIa	3300, 3050, 2966, 2915, 2880, 2825, 1610, 1585, 1520, 1345, 1328, 1144, 810, 775
IIIb	3288, 3060, 2922, 2858, 1700, 1598, 1527, 1325, 1303,1139, 821, 775
IIIc	3292, 3035, 2925, 2842, 1602, 1595, 1520, 1349, 1310,1128, 830, 808, 795, 780
IIId	3275, 3050, 2968, 2920, 2870, 2832, 1620, 1610, 1541, 1348, 1320, 1165, 802, 772
IIIe	3290, 3040, 2960, 2910, 2875, 2840, 11631, 1605, 1528, 1490, 1341, 1302, 1157, 820, 778
IIIf	3287, 3018, 2964, 2921, 2871, 2846, 1623, 1590, 1521, 1510, 1328, 1319, 1148, 805, 776
IIIg	3332, 3035, 2960, 2922, 2868, 2852, 1642, 1608, 1542, 1506, 1325, 1321, 1168, 820, 776
IIIh	3327, 3031, 2981, 2941, 2847, 1652, 1604, 1538, 1498, 1352, 1313, 1240, 1164, 1045, 832, 772
IIIi	3315, 3041, 2978, 2934, 2838, 1647, 1620, 1522, 1510, 1359, 1318, 1225, 1171, 1035, 815, 762
IIIj	3441, 3308, 3025, 2968, 2919, 2872, 2857, 1638, 1612, 1548, 1338, 1297, 1154, 818, 767
IIIk	3455, 3321, 3042, 2922, 2853, 1628, 1598, 1520, 1506, 1354, 1318, 1162, 816, 764
IIIl	3458, 3311, 3031, 2933, 2858, 1641, 1618, 1552, 1510, 1328, 1294, 1168, 828, 803, 779

*IR of tetrazolium salts are almost similar to their corresponding formazan the only discernible difference is disappearance of -NH group in the range of 3350-3275 cm^{-1}.

Conclusion

Most of the synthesized formazan and their tetrazolium salts have shown good to moderate antibacterial activity. The structure of all the compounds were confirmed by IR , PMR spectral data and are further supported by elemental analysis.

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