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Research Article

Synthesis, Characterisation and Antimicrobial Activity of 1-Isonicotinoyl -3-(4-Methoxy Phenyl)-5-(Substituted Phenyl) Formazanes

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ABSTRACT

Synthesis of1-isonicotinoyl -3-(4-methoxy phenyl)-5-(substituted phenyl) formazanes **2a-n**have been achieved first reacting 4-methoxy benzaldehyde with isoniazide gave the N'-(methoxybenzylidene) isonicotinohydrazide **1.**Theisonicotinohydrazide on treatment with diazotised aromatic amine in pyridine medium furnished formazanes. The formazanes were screened for their antimicrobial activity.

Keywords:isoniazide, isonicotinohydrazide, formazanes.

INTRODUCTION

Water insoluble pigments known as formazanes[1, 2]Tetrazoline salts obtained after reduction of formazanes have used as test reagent for growing plant and germinated seeds[3-6].The stability of coloured formazanes and their insolubility in water suggested the possibility of using tetrazoline salts as hydrogen acceptors in developing histochemical method for demonstrating a variety of enzymes with aid of suitable substrates. Formazanes are used as dyes[7-8] and belong to azo dye[8] family and as chelating agents[9]. Antiviral[10], antimicrobial[11] and anti-inflammatory[12] activities of formazanes have been reported in literature. Some formazanes were assessing their antiviral, anticancer activity[13, 14] and anti-HIV activities[15].

EXPERIMENTAL

Melting points were determined by open capillary method and uncorrected. IR spectra (cm^{-1}) were recorded on Perkin Elmer spectrophotometer in KBr pellets. ^1H NMR spectra were recorded on Bruker – 400 MHzFT-NMR using TMS as internal reference. The elemental analysis was found satisfactory; purity of compounds was checked by TLC on silica gel plates.

Synthesis of N'-(4-methoxy benzylidene) isonicotinohydrazide:

Solution of 4-methoxy benzaldehyde (0.01mol) in ethanol (5ml) and isoniazide (0.01mol) in ethanol (5ml) were mixed and the mixture refluxed for 30 min[16]. On cooling the liquid hydrazone separated as light yellow solid. It was filtered under suction and recrystallised from ethanol, M.P. 124 ^{0}C, yield-70%.

Synthesis of 1-isonicotinoyl -3-(4-methoxy phenyl)-5-(substituted phenyl) formazanes:

Primary amine (0.001mol)wasdissolvedinaqueous hydrochloric acid (4ml, 1:1).The content were cooled and aqueous sodium nitrate (0.3gm in 2ml water) was slowly added, N'-(4 methoxy bezylidene) isonicotinohydrazide (0.01mol) was dissolved in dry pyridine (10ml) and sodium acetate (0.3gm) was added.The contents were cooled in ice bath and stirred.The solution of benzene diazonium chloride was added drop wise for 30 mins; maintaining low temperature (0^0C).The reaction mixture kept in an ice bath for 4 hrs.And then poured with stirring in ice water. The resulting solid was washed with water till free from pyridine, filtered under suction and dried. The product was crystallised from ethanol.

1-isonicotinoyl-3-(4-methoxyphenyl)-5-phenylformazan; 2a: Colour- Light Yellow; yield 70%; m.p.118-120^{0}C, IR (KBr):1033.8-1176.5 (C-O-C), 1508.2 (-N=N-), 1596.9 (-C=N), 1670.2 (>C=O), 3448.5 (-N-H). Anal. Calculated for $C_{20}H_{17}N_5O_2$: C, 60.84; H, 4.77; N, 19.49 Found: C, 60.78; H, 4.69; N, 19.41.

5-(2-chlorophenyl)-1-isonicotinoyl-3-(4-methoxyphenyl)formazan; 2b: Colour- Brown; yield 68.94 %; m.p. 132-133^{0}C. IR (KBr): 1033.8-1172.6 (C-O-C), 1415.7 (-N=N-), 1600.8 (-C=N), 1678 (>C=O), 3444.6 (-N-H).Anal. Calculated for $C_{20}H_{16}ClN_5O_2$: C, 60.99; H, 4.09; N, 17.78 Found: C, 60.88; H, 3.99; N, 17.81.

5-(3-chlorophenyl)-1-isonicotinoyl-3-(4-methoxyphenyl) formazan; 2c: Colour- Brown; yield 62.61 %; m.p. 122-124^{0}C. IR (KBr): 1033.8-1172.6 (C-O-C), 1411.8 (-N=N-), 1560 (-C=N), 1674 (>C=O), 3448.5 (-N-H).Anal. Calculated for $C_{20}H_{16}ClN_5O_2$: C, 60.99; H, 4.09; N, 17.78 Found: C, 60.88; H, 3.99; N, 17.81.

5-(4-chlorophenyl)-1-isonicotinoyl-3-(4-methoxyphenyl) formazan; 2d: Colour- Brown; yield 73.41 %; m.p. 82-83^{0}C. IR (KBr):1029.9-1176.5 (C-O-C), 1496.7 (-N=N-), 1604.7(-C=N), 1678 (>C=O), 3452.3 (-N-H). ^{1}H-NMR (DMSO-d^6): δ, 3.8 (s,3H,OCH_3), 9.8 (s,1H,N- H), 8.7-8.3 (m,4H,-pyridine), 7.2-6.9 (m,4H, -C_7H_7O),7.6-7.4 (m,4H, -C_6H_4Cl) . Anal. Calculated for $C_{20}H_{16}ClN_5O_2$: C, 60.99; H4.09; N17.78 Found: C, 60.88; H, 4.00; N, 17.74.

1-isonicotinoyl-3-(4-methoxyphenyl)-5(2-nitrophenyl)formazan; 2e: Colour- Deep Brown; yield 60.54%; m.p. 130-132^{0}C. IR (KBr): 1033.8-1168.8 (C-O-C), 1515.9 (-N=N-), 1604.7(-C=N), 1678 (>C=O), 3452.3 (-N-H). Anal.Calculated for $C_{20}H_{16}N_6O_4$: C, 59.40; H, 3.99; N, 20.78. Found: C, 59.34; H, 3.91; N, 20.81.

1-isonicotinoyl-3-(4-methoxyphenyl)-5(3-nitrophenyl) formazan; **2f:** Colour- Brown; yield 62.76%; m.p. 134-135^{0}C. IR (KBr): 1037.6-1176.5 (C-O-C), 1427.2 (-N=N-), 1568 (-C=N), 1672.2 (>C=O), 3403 (-N-H). ^{1}H-NMR ($CDCl_3$), δ, 3.8 (s,3H,OCH_3), 9.8 (s,1H,N- H), 8.7-8.2 (m,4H,-pyridine), 7.4-7.2 (m,4H, - C_7H_7O),7.8-7.5 (m,4H, -$C_6H_4NO_2$); Anal. Calculated for $C_{20}H_{16}N_6O_4$: C, 59.40; H, 3.99; N, 20.78. Found: C, 59.34; H, 3.91; N, 20.81.

1-isonicotinoyl-3-(4-methoxyphenyl)-5(4-nitrophenyl) formazan; 2g: Colour-Yellow; yield 60.30 %; m.p. 130-131^{0}C. IR (KBr): 1033.8-1168.8 (C-O-C), 1515.9 (-N=N-), 1604.7(-C=N), 1678 (>C=O), 3452.3 (-N-H). Anal.Calculated for $C_{20}H_{16}N_6O_4$: C, 59.40; H, 3.99; N, 20.78. Found: C, 59.42; H, 3.91; N, 20.84.

1-isonicotinoyl-3-(4-methoxyphenyl)-5-o-tolylformazan; 2h: Colour- Deep Brown; yield 65.60%; m.p. 132-134^{0}C. IR (KBr): 1033.8-1168.8 (C-O-C), 1515.9 (-N=N-), 1600.8 (-C=N), 1670.2 (>C=O), 3444.6 (-N-H). Anal.Calculated for $C_{21}H_{19}N_5O_2$: C, 67.55; H, 5.13; N, 18.76. Found: C, 67.49; H, 5.00; N, 18.82.

1-isonicotinoyl-3-(4-methoxyphenyl)-5-p-tolylformazan; 2i: Colour- Light Yellow; yield 70.42%; m.p. 122-123^{0}C. IR (KBr): 1033.8-1176.5 (C-O-C), 1415.7 (-N=N-), 1608.5 (-C=N), 1678 (>C=O), 3452.3 (-N-H).^{1}H-NMR($CDCl_3$), δ, 3.8 (s,3H,OCH_3), 2.3 (s,3H,-CH_3) 9.8 (s,1H,N- H), 8.7-8.5 (m,4H,-pyridine), 7.1-6.9 (m,4H, -C_7H_7),7.3-7.2 (m,4H, - C_7H_7O) . Anal.Calculated for $C_{21}H_{19}N_5O_2$: C, 67.55; H, 5.13; N, 18.76. Found: C, 67.58; H, 5.00; N, 18.71.

1-isonicotinoyl-5-(2-methoxyphenyl)-3-(4-methoxyphenyl) formazan; 2j: Colour- Deep Brown; yield 73.50%; m.p. 123-125^{0}C. IR (KBr): 1029.9-1172.6 (C-O-C), 1485.1 (-N=N-), 1596.9 (-C=N), 1666.4 (>C=O), 3448.5 (-N-H). ^{1}H-NMR ($CDCl_3$), δ, 3.8 (s, 3H, OCH_3), 4.2 (s, 3H,-OCH_3) 9.8 (s, 1H, N-H), 8.7-8.0 (m, 4H, -pyridine), 7.2-6.9 (m, 4H, - C_7H_7O), 7.8-7.6 (m, 4H, - C_7H_7O)Anal. Calculated for $C_{21}H_{19}N_5O_3$: C, 64.77; H, 4.92; N, 17.98. Found: C, 64.82; H, 4.98; N, 18.10.

1-isonicotinoyl-3, 5- bis (4-methoxyphenyl) formazan; 2k: Colour- Deep Brown; yield 75.66%; m.p. 126-128^{0}C. IR (KBr): 1033.8-1172.6 (C-O-C), 1415.7 (-N=N-), 1508.2 (-C=N), 1674.1 (>C=O), 3456.2 (-N-H). Anal.Calculated for $C_{21}H_{19}N_5O_3$: C, 64.77; H, 4.92; N, 17.98. Found: C, 64.71; H, 4.88; N, 18.89.

4-(((2-isonicotinoylhydrazono)(4-methoxyphenyl)methyl)diazenyl)benzene-sulfonicacid;2l: Colour-White; yield 53.94%; m.p. 134-136^{0}C. IR (KBr): 1033.8-1168.8 (C-O-C), 1515.9 (-N=N-), 1600.8 (-C=N), 1670.2 (>C=O), 3444.6 (-N-H).^{1}H-NMR (DMSO-d^6), δ,3.8 (s,3H,OCH_3), 9.8 (s,1H,N- H), 11.8 (s,1H,-SO_3H), 8.7-8.3 (m,4H,-pyridine), 7.0-6.9 (m,4H, - C_7H_7O)7.7-7.6 (m,4H, -$C_6H_5SO_3$). Anal.Calculated for $C_{20}H_{17}N_5O_5S$: C, 54.66; H, 3.99; N, 15.94. Found: C, 54.62; H, 3.91; N, 15.88.

4-(((2-isonicotinoylhydrazono) (4-methoxyphenyl) methyl) diazenyl) benzonicacid;2m: Colour-White; yield 52.64%; m.p. 140-142^{0}C. IR (KBr): 1033.8-1172.6 (C-O-C), 1485.1 (-N=N-), 1596 (-C=N), 1678 (>C=O), 3448 (NH), 3200 (-OH). Anal.Calculated for $C_{21}H_{17}N_5O_4$: C, 62.53; H, 4.25; N, 17.36. Found: C, 62.67; H, 4.18; N, 17.41.

4-(((2-isonicotinoylhydrazono) (4-methoxyphenyl) methyl) diazenyl) naphthalene-1-sulfonic-acid;2n: Colour-White; yield 44.36%; m.p. 138-140^{0}C. IR (KBr): 1033.8-1168.8 (C-O-C), 1515.9 (-N=N-), 1604.7 (-C=N), 1674 (>C=O), 3444.6 (-N-H). ^{1}H-NMR (DMSO-d6), δ, 3.8 (s, H,

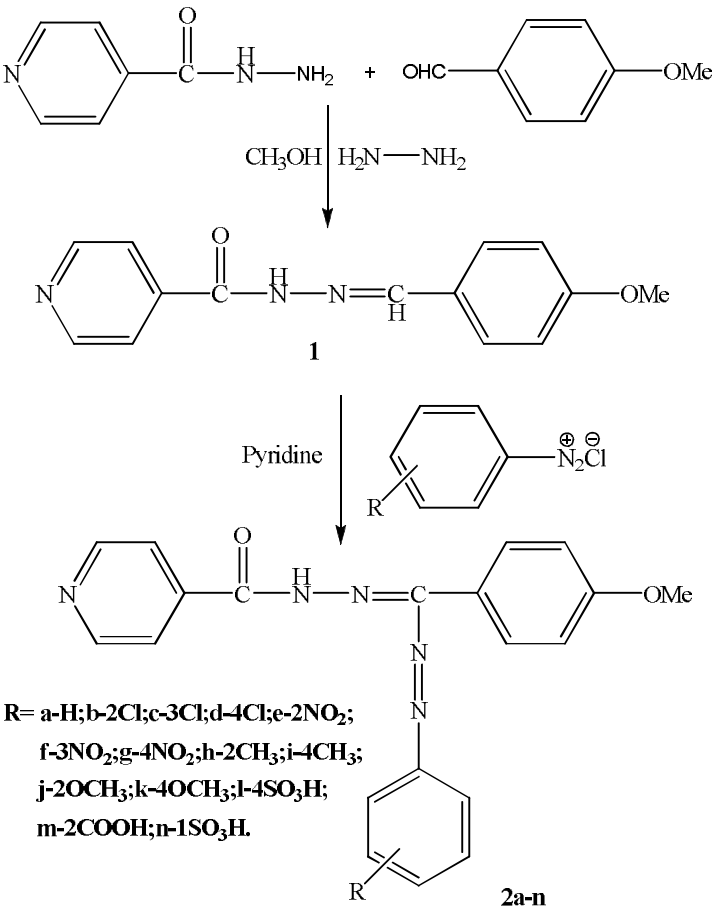
OCH_3), 9.8 (s, 1H, N- H), 11.8 (s, 1H,-SO_3H), 8.7-8.3 (m, 4H, -pyridine), 7.0-6.9 (m, 4H, - C_7H_7O)7.7-7.6 (m, 6H, -$C_{10}H_7SO_3$). Anal.Calculated for $C_{24}H_{19}N_5O_5S$: C, 58.89; H, 3.91; N, 14.31. Found: C, 58.78; H, 3.89; N, 14.40.

Biological testing of compounds

The synthesized compounds **2a-n** were evaluated in-vitro for antimicrobial activity against *Escherichia coli, Bacillus, Salmonella thphimurium, A.niger* at the concentration 1mg/ml by paper disc diffusion method using DMF as solvent and nutrient agar was employed as culture media. The result were obtained in the form of cleaning zone and were noted after the period of incubation (at 37^0C for 24-48 hrs).The zone of inhibitions were measured in mm and the data is presented in table-I.

RESULTS AND DISCUSSION

In the present investigation the intermediate N'-(4-methoxy benzylidene)isonicotinohydrazide was obtained by the reaction of isoniazide with p-methoxybenzaldehyde. The N'-(4-methoxy benylidene) isonicotinohydrazide on treatment with diazotised aromatic amines in pyridine medium furnished formazanes. The reaction sequence has been obtained in Scheme-1.



1

2a-n

SCHEME-I

Table 1

Compound	Zone of inhibition in mm			
	Bacillus	***E-coli***	***Salmonella***	***A.niger***
IIa	**N**	**6**	**N**	**N**
IIb	**N**	**10**	**N**	**N**
IIc	**N**	**7**	**N**	**N**
IId	**N**	**7**	**N**	**N**
IIe	**N**	**7**	**N**	**N**
IIf	**N**	**7**	**7**	**N**
IIg	**N**	**7**	**9**	**N**
IIh	**N**	**7**	**7**	**N**
IIi	**N**	**8**	**6**	**N**
IIj	**N**	**7**	**6**	**N**
IIk	**N**	**7**	**N**	**N**
IIl	**N**	**8**	**N**	**N**
IIm	**N**	**7**	**7**	**N**
IIn	**N**	**7**	**6**	**N**

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