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Synthesis, Characeterization and *in vitro* antibacterial activity of novel 3-(4-methoxyphenyl)-1-isonicotinoyl-5-(substituted phenyl)-formazans

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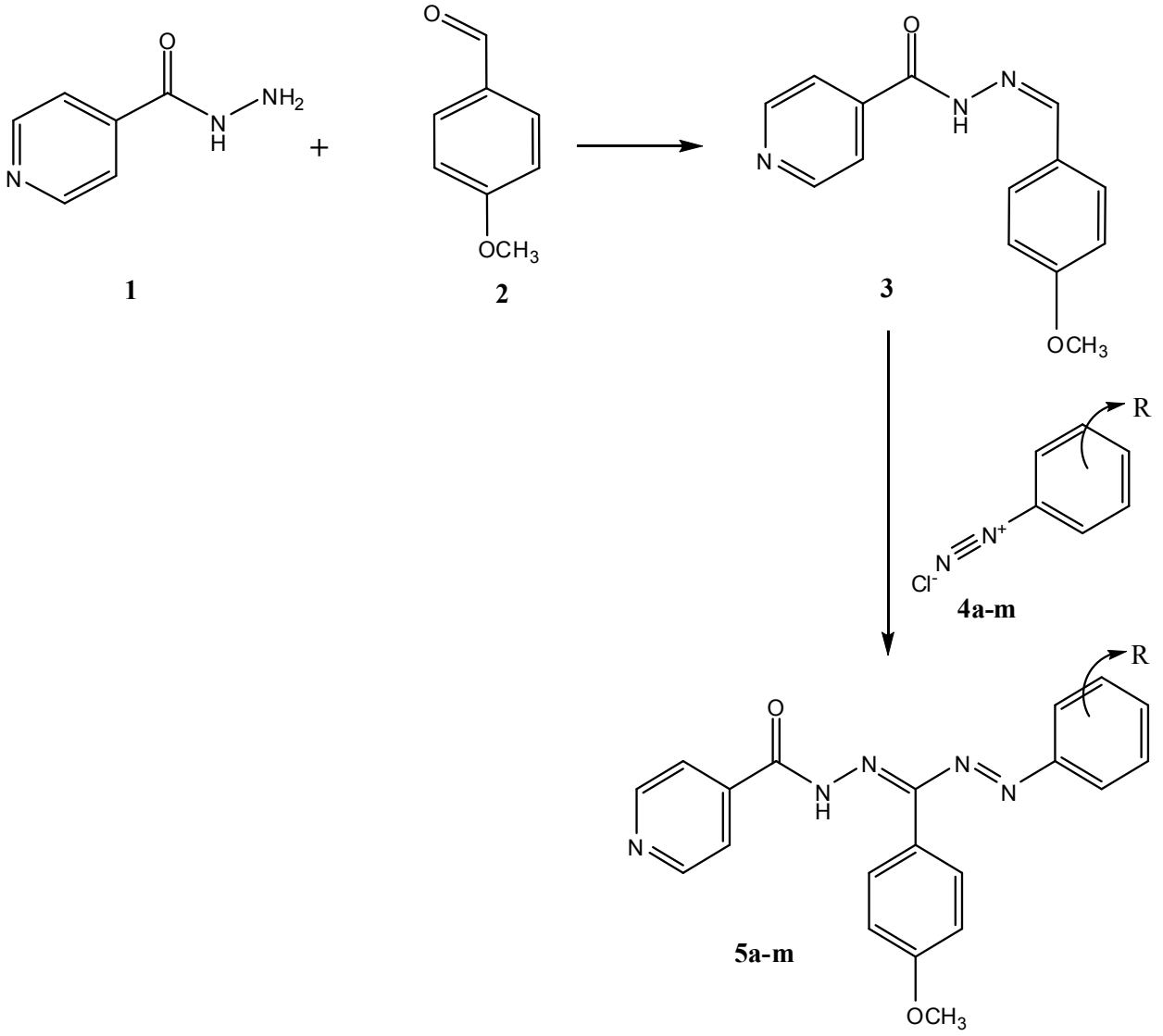
Abstract: Formazans **5a-m** have been prepared by the condensation of Schiff base **3** and diazonium salt of substituted aromatic amines, **4a-m**. The intermediate Schiffbase, **3** was itself synthesized by condensation of isonicotinic acid hydrazide, **1** with p-methoxybenzaldehyde, **2**. The structures of the compound have been confirmed by elemental analysis and spectral analysis. The antibacterial activity of the compounds has also been screened.
Keywords: isonicotic acid hydrazide, Schiff base, formazans, antibacterial activity.

Introduction

Each year there are 8.7 million new cases of TB and estimated 1.7 million deaths. If current control efforts are not massively expanded, TB will kill more than 40 million people over the next 25 years. The existing recommended TB treatment is based on 2 months of four drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) followed by 4 months of two drugs (isoniazid and rifampicin). But the current recommended strategy is facing two problems: multidrug resistance and HIV/AIDS pandemic[1,2]. Additionally, in patients with impaired cellular immunity (HIV-syndrom), mycobacterial and fungal (Aspergillus, Histoplasma, etc.) infections predominate and may coexist[3]. Thus, there are two basic approaches to get a new drug for TB: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment; and (ii) searching novel structures, that the TB organism 'has never seen before', for the treatment of multidrug-resistant TB[4].

Also, during the last few years the potential of Schiff base derivatives in pharmaceutical and medicinal field have been subjected to investigation. Literature survey reveals that Schiff base derivatives are associated with number of pronounced antibacterial activities[5-8] against gram positive (B. subtilis, B. sphaericus, S. aureus etc) and gram negative organism (E. coli, K. aerogenes, P. aeruginosa etc). Furthermore, Schiff bases are utilized as starting material in the synthesis of pharmaceutically important compounds such as formazans[9] derivatives which have achieved considerable attention in analytical chemistry[10] because of their high sensitivity toward many metals and organometals, but many authors have shown that this class of compounds is active against the Ranikhet disease virus[11] as well as against the plant viruses TMV[12] (*Tobacco mosaic virus*) and GMV[12] (*gomphrena mosaic virus*). They are also well known to have antiviral[13], anti-inflammatory-analgesic[14], antifertility[15], antimicrobial[16-19] and activities. Moreover, they have been studied extensively because of their ready accessibility, diverse chemical reactivity, and broad spectrum of biological activities & wide range of applications.

To randomly explore[20-24] the novel compounds for better pharmacological activity, our idea was to combine isoniazid and various aromatic amines in one single molecule to get formazan derivatives.**(Scheme -1)**.



Scheme 1: Synthetic route to formazan derviatives

Experimental

All the melting points were taken in open capillaries tube and are uncorrected. The purity of compounds was checked routinely by TLC (0.5 mm thickness) using silica gel – G coated Al – plates (Merck) and spots were visualized by exposing the dry plates in iodine vapours. IR spectra (ν_{max} in cm^{-1}) were recorded on Shimadzu FTIR spectrophotometer using KBr or Nujol technique.^{1}H & ^{13}C NMR spectra on a Bruker's WM 400 FT MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The elemental analysis (C, H, N) of compounds was performed on Carlo Erba – 1108 elemental analyzer.

N'-(4-methoxybenzylidene)isonicotinohydrazide, 3

N'-(4-methoxybenzylidene)isonicotinohydrazide, **3** was prepared by reaction between the 4-methoxybenzaldehyde**, 2** (0.01 mole) with isonicotinic acid hydrazide (0.01 mole) in ethanol/H_2O following the procedure described in reported literature[19]. The solid product thus generated was filtered, wash with dry ether and crystallized from absolute alcohol. Yield: 70%; m.p.: 160-162^{o}C. IR ν_{max} in cm^{-1}: 3340(NH) and 1339(CN), 1665(CO), 1625(N=CH), 3023 & 1510(aromatic ring, CH & C=C), 1256(OCH_3). ^{1}H NMR δppm($CDCl_3$): 3.50(s, 1H, OCH_3), 8.86(s,1H,N=CH), 8.00(s,1H,CONH), 6.92-7.70(m, 4H, ArH). ^{13}C NMR (125 MHz, DMSO-d6) δ ppm: 165.7; 150.2, 150.2;

140.2; 129.1; 111.1; 120.1; 120.1; 136.6; 111.1; 130.7; 161.0; 145.6, 59.8.

3-(4-methoxyphenyl)-1-isonicotinoyl-5-phenylformazan 5a

Aniline, **4a** (0.01 mole) in aqueous HCl(10ml) was reacted with N'–(4–methoxybenzylidene)isonicotinohydrazide (0.01 mole) following the procedure described in reported literature[19]. The dark coloured product obtained was crystallized from ethanol. Yield 83%, mp 144~45°C. IR(ν_{max} in cm^{-1}): 3310(NH) &1330 (CN), 1680(CO) 1610(N=CH), 1590(N=N), 1256(OCH_3). ^{1}H NMR($CDCl_3$) δ ppm: 3.81(s, 3H, OCH_3), 12.15 (s, 1H, CONH, D_2O exchangeable), 7.79-8.10(m,13H, ArH). **^{13}C NMR δ ppm**:114.96-150.67(Ar-C), 151.56(N=C), 163.25(CO), 55.79(OCH_3).

Other compounds, **5b-j** were prepared in similar manner and characterization data for different substituted formazans are given in **Table – 1.**

Selected spectral data of the products, 5b-j.

5b: IR (ν_{max} in cm^{-1}):1680(CO), 3310(NH) &1330(CN), 1610(N=C of Schiff base), 1590(N=N of formazans), 1258(OCH_3). **^{1}H NMR δ ppm** :12.20(s, 1H, CONH-, D_2O exchangeable), 7.77-8.14(m, 12H, Ar-H), 3.79(s, 6H, 2xOCH_3),. **^{13}C NMR δ ppm**: 114.55-152.18(Ar-C), 151.56(N=C), 163.25(CO), 55.81 & 59.12(OCH_3).

5c: IR (ν_{max} in cm^{-1}):1680(CO), 3310(NH) &1330(CN), 1610(N=C of Schiff base), 1580(N=N of formazans), 1258(OCH_3). **^{1}H NMR δ ppm** :12.15(s, 1H, CONH-, D_2O exchangeable), 7.78-8.15(m, 12H, Ar-H), 3.71(s, 6H, 2xOCH_3),. **^{13}C NMR δ ppm**: 114.66-150.78(Ar-C), 151.79(N=C), 163.25(CO), 58.92 & 59.12(OCH_3).

5d: IR (ν_{max} in cm^{-1}):1690(CO), 3320(NH) &1330(CN), 1610(N=C of Schiff base), 1585(N=N of formazans), 1258(OCH_3). **^{1}H NMR δ ppm** :12.18(s, 1H, CONH-, D_2O exchangeable), 7.92-8.18(m, 12H, Ar-H), 3.72(s, 6H, 2xOCH_3),. **^{13}C NMR δ ppm**: 114.66-150.79(Ar-C), 151.76(N=C), 163.21(CO), 59.14(2xOCH_3).

5e: IR (ν_{max} in cm^{-1}):1690(CO), 3330(NH) &1320(CN), 1610(N=C of Schiff base), 1570(N=N of formazans), 1518(NO_2), 1256(OCH_3). **^{1}H NMR δ ppm** :12.10(s, 1H, CONH-, D_2O exchangeable), 7.81-8.10(m, 12H, Ar-H), 3.72(s, 1H, OCH_3),. **^{13}C NMR δ ppm**: 114.81-150.67(Ar-C), 151.56(N=C), 163.25(CO), 59.11(OCH_3).

5f: IR (ν_{max} in cm^{-1}):1695(CO), 3335(NH) &1325(CN), 1610(N=C of Schiff base), 1575(N=N of formazans), 1518(NO_2), 1256(OCH_3). **^{1}H NMR δ ppm** :12.20(s, 1H, CONH-, D_2O exchangeable), 7.89-8.20(m, 12H, Ar-H), 3.79(s, 1H, OCH_3),. **^{13}C NMR δ ppm**: 114.72-150.77(Ar-C), 150.56(N=C), 163.25(CO), 59.20(OCH_3).

5g: IR (ν_{max} in cm^{-1}):1690(CO), 3330(NH) &1325(CN), 1610(N=C of Schiff base), 1575(N=N of formazans), 1518(NO_2), 1256(OCH_3). **^{1}H NMR δ ppm** :12.19(s, 1H, CONH-, D_2O exchangeable), 7.91-8.20(m, 12H, Ar-H), 3.70(s, 1H, OCH_3),. **^{13}C NMR δ ppm**: 114.95-150.67(Ar-C), 150.51(N=C), 163.25(CO), 60.10(OCH_3).

5h: IR (ν_{max} in cm^{-1}):1692(CO), 3335(NH) &1320(CN), 1610(N=C of Schiff base), 1570(N=N of formazans), 1256(OCH_3), 810(C-Cl). **^{1}H NMR δ ppm** :12.10(s, 1H, CONH-, D_2O exchangeable), 7.89-8.14(m, 12H, Ar-H), 3.72(s, 1H, OCH_3),. **^{13}C NMR δ ppm**: 114.95-151.77(Ar-C), 155.56(N=C), 164.19(CO), 60.22(OCH_3).

5i: IR (ν_{max} in cm^{-1}):1694(CO), 3335(NH) &1325(CN), 1610(N=C of Schiff base), 1580(N=N of formazans), 1256(OCH_3), 810(C-Cl). **^{1}H NMR δ ppm** :12.05(s, 1H, CONH-, D_2O exchangeable), 7.77-8.18(m, 12H, Ar-H), 3.74(s, 1H, OCH_3),. **^{13}C NMR δ ppm**: 114.86-151.89(Ar-C), 155.18(N=C), 164.25(CO), 60.92(OCH_3).

5j: IR (ν_{max} in cm^{-1}):1695(CO), 3335(NH) &1325(CN), 1610(N=C of Schiff base), 1580(N=N of formazans), 1256(OCH_3), 810(C-Cl). **^{1}H NMR δ ppm** :12.05(s, 1H, CONH-, D_2O exchangeable), 7.72-8.19(m, 12H, Ar-H), 3.77(s, 1H, OCH_3),. **^{13}C NMR δ ppm**: 114.95-151.77(Ar-C), 155.16(N=C), 164.21(CO), 60.72(OCH_3).

5k: IR (ν_{max} in cm^{-1}):1690(CO), 3340(NH) &1320(CN), 1610(N=C of Schiff base), 1580(N=N of formazans), 2928(CH_3), 1256(OCH_3). **^{1}H NMR δ ppm** :12.95(s, 1H, CONH-, D_2O exchangeable), 7.92-8.27(m, 12H, Ar-H), 3.77(s, 1H, OCH_3), 2.74(CH_3). **^{13}C NMR δ ppm**: 114.95-155.87(Ar-C), 150.57(N=C), 163.24(CO), 60.9(OCH_3), 24.01(CH_3).

5l: IR (ν_{max} in cm^{-1}):1695(CO), 3340(NH) &1325(CN), 1610(N=C of Schiff base), 1580(N=N of formazans), 2928(CH_3), 1256(OCH_3). **^{1}H NMR δ ppm** :12.97(s, 1H, CONH-, D_2O exchangeable), 7.77-8.29(m, 12H, Ar-H), 3.71(s, 1H, OCH_3), 2.77(CH_3). **^{13}C NMR δ ppm**: 114.96-155.87(Ar-C), 150.56(N=C), 163.25(CO), 60.7(OCH_3), 24.02(CH_3).

5m: IR (ν_{max} in cm^{-1}):1695(CO), 3340(NH) &1325(CN), 1610(N=C of Schiff base), 1580(N=N of formazans), 2928(CH_3), 1256(OCH_3). **^{1}H NMR δ ppm** :12.91(s, 1H, CONH-, D_2O exchangeable), 7.77-8.29(m, 12H, Ar-H), 3.79(s, 1H, OCH_3), 2.71(CH_3).**^{13}C NMR δ ppm**: 114.95-155.87(Ar-C), 150.54(N=C), 163.24(CO), 60.9(OCH_3), 24.03(CH_3).

Antibacterial activity

The minimum inhibitory concentrations (MIC) of various substituted formazans were tested against representative Gram-positive organisms viz. *Bacillus subtilis (MTCC 121), Micrococcus lucteus(MTCC 106), Bacillus sphaericus (MTCC 11) Staphylococcus aureus (MTCC 96) and three Gram-negative organisms viz. Chromobacterium violaceum (MTCC 2656), Klebseilla aerogenes (MTCC 39), Pseudomonas aeruginosa (NTCC 791), Escherichia coli (MTCC 443), klebseilla pneumonial (MTCC 109), Salomonella paratyphi A (MTCC 735)* by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards[25]. Standard antibacterial agents like penicillin and streptomycin were also screened under identical conditions for comparison. The minimum inhibitory concentration (MIC) values are presented in **Table 2.**

Table – 1: Characterization data of 3 and 4b-j.

Compd.	R	Melting point(^{O}C)	Yield (%)	UV (DMF) λmax in nm	Anal. Calcd. (found)/ %		
					C	H	N
3	-	107 ~ 09	70 ~ 71	310	64.19 (64.29)	4.14 (4.18)	17.28 (17.38)
5a	H	144 ~ 45	69 ~ 71	282	66.84 (66.92)	4.77 (4.82)	19.49 (19.34)
5b	2-OCH_3	142 ~ 44	72 ~ 74	371	64.77 (64.82)	4.92 (4.87)	17.98 (17.77)
5c	3-OCH_3	146 ~ 47	81 ~ 82	372	64.77 (64.72)	4.92 (4.90)	17.98 (17.79)
5d	4-OCH_3	149 ~ 50	77 ~ 79	379	64.77 (64.76)	4.92 (4.87)	17.98 (17.89)
5e	2-NO_2	129 ~ 31	69 ~ 71	275	59.40 (59.37)	3.99 (3.89)	20.78 (20.89)
5f	3-NO_2	134 ~ 36	62 ~ 67	272	59.40 (59.67)	3.99 (3.79)	20.78 (20.81)
5g	4-NO_2	127 ~ 29	69 ~ 71	277	59.40 (59.43)	3.99 (3.77)	20.78 (20.84)
5h	2-Cl	136 ~ 38	70 ~ 73	302	60.99 (60.79)	4.09 (4.09)	17.78 (17.79)
5i	3-Cl	139 ~ 40	76 ~ 77	303	60.99 (60.79)	4.09 (4.11)	17.78 (17.91)
5j	4-Cl	148 ~ 49	79 ~ 81	307	60.99 (60.82)	4.09 (4.18)	17.78 (17.92)
5k	2-CH_3	130 ~ 33	80 ~ 82	281	67.55 (67.67)	5.13 (5.18)	18.76 (18.82)
5l	3-CH_3	135 ~ 36	84 ~ 86	286	67.55 (67.72)	5.13 (5.21)	18.76 (18.78)
5m	4-CH_3	139 ~ 40	87 ~ 89	287	67.55 (67.77)	5.13 (5.18)	18.76 (18.96)

Results and Discussion

It has been observed that the test compounds **(3, 5a-j)** exhibited interesting antibacterial activity, however with a degree of variation. Compound **5a, 5d, 5h** & **5k** exhibited the highest degree of inhibition against all tested gram +ve organisms, while compound **5c** & **5h** showed highest degree of inhibition against all tested gram –ve organisms. Compounds **5c, 5d, 5h** and **5k** showed highest degree of inhibition against *Escherichia coli, Klebseilla pneumonial, Salomonella paratyphi A,* while compound **5k** showed highest inhibition against *Chromobacterium violaceum, Klebseilla aerogenes, Pseudomonas aeruginosa, Escherichia coli* respectively

.**Table – 2. *In vitro* antibacterial activity of 3 & substituted formazans, 5b-j.**

Compd.	MIC, µg/mL									
	Gram +ve organism				Gram –ve organism					
	B.s	***M.l***	***B.sph***	***S.a***	***C.v***	***K.a***	***P.a***	***E.c***	***K.p***	***S.p***
3	25	25	25	20	25	-	25	20	20	25
5a	12.5	12.5	12.5	12.5	25	20	20	20	20	25
5b	20	20	20	12.5	20	20	20	20	12.5	25
5c	25	25	20	12.5	12.5	12.5	12.5	12.5	12.5	12.5
5d	12.5	12.5	12.5	12.5	25	25	20	12.5	12.5	12.5
5e	25	12.5	20	12.5	20	20	-	20	-	25
5f	-	20	20	12.5	20	20	20	-	-	25
5g	25	25	-	25	-	25	20	-	20	25
5h	12.5	6.25	12.5	6.25	12.5	12.5	12.5	12.5	12.5	12.5
5i	20	25	25	25	20	20	20	25	-	-
5j	20	25	25	20	25	25	25	25	-	20
5k	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	20	20
5l	20	20	25	25	25	25	20	12.5	25	20
5m	20	25	25	25	20	20	20	20	25	25
Penicillin	1.56	3.12	3.12	1.56	12.5	6.25	12.5	6.25	6.25	12.5
Strepto-mycin	6.25	12.5	6.25	6.25	3.12	1.56	3.12	1.56	1.56	3.12

Gram +ve Organisms	*Gram -ve Organisms*
B.s: : Bacillus subtilis (MTCC 121),	*C.v. : Chromobacterium violaceum (MTCC 2656),*
M.l.: : Micrococcus lucteus(MTCC 106),	*K.a. : Klebseilla aerogenes (MTCC 39),*
B.sph.: Bacillus sphaericus (MTCC 11)	*P.a. :Pseudomonas aeruginosa (MTCC 791),*
S.a. : Staphylococcus aureus (MTCC 96)	*E.c. : Escherichia coli (MTCC 443),*
	K.p. : Klebseilla pneumonial (MTCC 109),
	S.p. : Salomonella paratyphi A (MTCC 735)

Conclusion

Ten Formazans derivatives were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structures thought of. Antibacterial activity of these compounds indicated that compound **5a, 5d, 5h** & **5k** were found to be showing comparable activity against some bacteria compared to standard antibiotic drugs.

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