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Synthesis of *N′*-Nicotinoyl Sulfonohydrazides and their Antimicrobial Activity

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Summary: *N′*-Nicotinoyl sulfonohydrazide derivatives **3-13** were synthesized from nicotinyl hydrazide and evaluated for their antimicrobial potential against Gram positive bacterial strains (*Bacillus cereus, Bacillus subtilis, Corynebacterium diphtheriae, Staphylococcus fecalis, Staphylococcus aureus, and MRSA* **(***Methicillin-resistant Staphylococcus aureus*)) and Gram negative bacterial strains (*Escherichia*.coli, *Pseudomonas aeruginosa, Salmonella ParatyphiB, Salmonella tyhpi*). Compound **13** showed outstanding antibacterial activity against *Staphylococcus fecalis* and compounds **7** and **13** were found to be moderately activite against *Salmonella Paratyphi B*, shown by their zone of inhibition values. In addition to that compond **9** also showed moderate activity against *Escherichia coli*. All derivatives **3-13** were also subjected for the evaluation of their antifungal activity against *Saccharomyces cerevisiae, Microsporum canin, Rhizopus, Aspergillus niger, Candida albicans*, and *Candida tropicalis*. Compound **13** showed promising antifungal activity against *Rhizopus sp*. and compounds **9** and **10** showed moderate antifungal potential against *Microsporum canis, Aspergillus niger*, and *Candida tropicalis*. Other molecules demonstrated weak zone of inhibitions.

Keywords: Synthesis; *N′*-nicotinoylsulfonohydrazide; Antibacterial; Antifungal activity; Zone of inhibition.

Introduction

Nicotinic acid belongs to vitamin B group. It has received great importance in the last decades due to its vital role in the cure of human diseases like pellagra. Nicotinic acid analogs have also been known for their diverse medicinal effects. The synthetic advancement of nicotinic acid and its derivatives by means of their structural alterations are well reported. The polymorphs of nicotinic acid and its derivatives also used as a drug because those have varying dissolution rates [1]. Nicotininc acid plays an important role in the human body in different metabolic processes like digestion, fat synthesis *etc*. It is an inhibitor of fat-mobilizing lipolysis in adipose tissue [2]. It is used as a lipid lowering drug since decades [3-6] and have useful application at various cardiovascular events [7]. Derivatives of nicotinic acid showed antiatherosclerotic activity by changing the composition of the plasma lipids. Nicotinic acid utilized in the prohibition and suspension of atherosclerotic processes [8].

Hydrazides have a covalent linkage between nitrogen of hydrazine (NH_2-NH_2) to a carbonyl group (C=O). This functionality found in many pharmaceutical drug and posseses huge diversity in their biological potential such as antibacterial, [9], antitubercular [10], antifungal [11], anticancer [12], anticonvulsant, antiinflammatory, antiviral, and antiprotozoal activities [13].

Antimicrobial agents are the compounds that are used to destroy microorganisms or to inhibit their growth. These antimicrobial agents are synthetic chemicals or natural products obtained from natural source and utilized in the treatment of various infections. These agents are functional to the living tissues of plants, animals, and humans in order to destroy (bactericidal) or obstruct the development (bacteriostatic) of infectious microorganisms and known as antibiotics [14].

Our group has reported many compounds having hydrazine moiety as significant antimicrobial agents [15-18] (Fig-1). Therefore, we intended to further evalute the antimicrobial activity of *N′*-hydrazide class. For this purpose, we synthesized *N′*-nicotinoyl sulfonohydrazides (**3-13**) and evaluated them for their antimicrobial potential.

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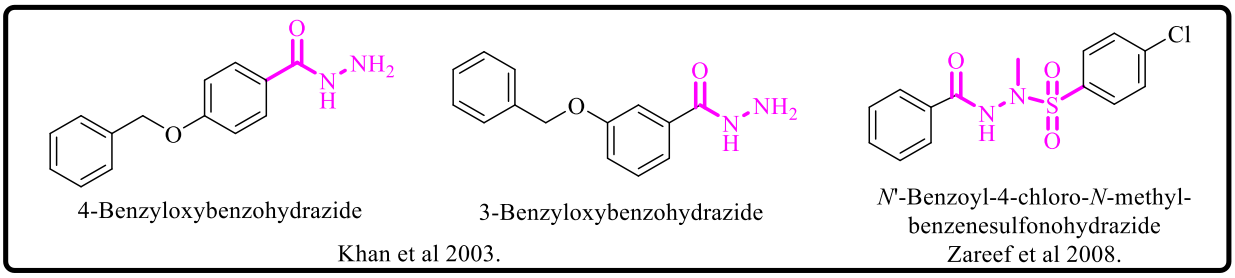


Fig-1: Previously identified lead molecules for antimicrobial activity

Experimental

Materials and methods

Thin layer chromatography (TLC) was performed on pre-coated silica gel aluminum plates (Kieselgel 60 F-254, 0.20 mm, Merck, Darmstadt, Germany). Chromatograms were visualized by using a handhold UV lamp at 254 and 365 nm or iodine vapour. ^{1}H-NMR spectroscopic analysis was performed on Advance Bruker AM spectrometers 400 MHz machine. Mass experiments were carried out on a Finnigan MAT-311A (Germany) mass spectrometer.

Bioassay

Antibacterial Assay

The antibacterial activity of synthesized compounds was determined by using agar disc diffusion method [19]. Saline solution (0.85%) was mixed with bacterial cultures (10 μL) and subjectedfor 24 h incubation. Turbidity of all samples was agreed with the standard inoculum of 0.5 Mac-Farland scale [~106 CFU/mL]. The Mueller Hinton agar (Oxoid) plates were seeded with all bacterial cultures grown in Mueller Hinton broth (Oxoid). All prepared discs containing test compounds were positioned on to the surfaces and plates were incubated for 24 h at 37 $^\circ$C. Zone of inhibition in mm showed the measurement of antibacterial activity. Antibacterial activity of all synthetic compounds was performed by using gentamicin as positive control. DMSO was used as negative control for antibacterial activity.

Antifungal activity

Antifungal activity was measured by disc diffusion method [19]. A small portion of fungal culture was shifted to normal saline 2-3 mL in a screw capped tube with few glass beads (diameter = 1 mm) and vortexes for about 10 min to prepare a homogeneous suspension fungal cultures. Sabouraud dextrose agar (SDA) plates were seeded with these fungal suspensions. Sterile filter discs containing stock solution 10 μL were positioned on to the surfaces. Plates were incubated for one week at room temperature antifungal activity of all synthetic compounds was performed using ketoconazole as positive control.

*Procedure for the synthesis of nicotinichydrazide (**2**)*

Nicotinic acid methyl ester **1** (5 g) and hydrazine monohydrate (10 mL) were taken in methanol (40 mL) into a round-bottommed flask (250 mL). Reaction mixture was refluxed for 24 h at 100 $^\circ$C. After evaporation of methanol, crystals of nicotinic hydrazide was obtained.

$C_7H_7NO_2$; White solid; Yield 85%; m.p. 162-164 $^\circ$C; ^{1}H-NMR (400 MHz, CD_3OD): δ 8.92 (s, 1H, H-2), 8.67 (m, 1H, H-5), 8.20 (m, 1H, H-6), 7.53 (m, 1H, H-5); EI MS: (rel. abund.%) *m/z*137 (61), 106 (100), 78 (100), 51 (31).

Procedure for the synthesis of N'-nicotinoylsulfonohydrazide **(3-13)**

Nicotinic hydrazide (1mmol) and triethylamine (1 mmol) were dissolved in 15 mL THF into a round-bottommed flask. Than, corresponding sulfonyl chloride (1.1 mmole) was added into the reaction mixture and refluxed for 48 h at 100 $^\circ$C. The progress of reaction was checked by TLC. After evaporation of solvent, solid product was extracted with ethyl acetate and water. Organic layer was dryied with sodium sulphate.

*N'-Nicotinyl-3-nitrobenzenesulfonohydrazide***(3)**

$C_{12}H_{10}N_4O_5S$; White solid; Yield: 62%; R_f = 0.87 (*n*-hexane: ethyl acetate 7:3); m.p. 228-230 $^\circ$C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.84 (s, 1H, H-2),

8.74 (m, 2H, H-2′, H-4′), 8.47 (m, 1H, H-5′), 8.30 (d, 1H, $J_{6',5'}$ = 7.6 Hz, H-6′), 8.20 (d, 1H, $J_{6,5}$= 8.0 Hz, H-6), 7.79 (t, 1H, $J_{5(4,6)}$ = 8.0 Hz, H-5), 7.63(m, 1H, H-4); ^{13}C-NMR (75 MHz, CD_3OD): δ_H 164.5, 148.2, 148.0, 147.5, 139.5, 134.2, 133.0, 130.1, 125.0, 123.2, 127.3, 129.7; EI-MS:(rel. abund.%), *m/z*322 (7), 292 (6), 242 (5), 187 (22), 171 (9), 157 (4), 139 (5), 123 (69), 78 (46), 50 (66); IR (KBr, cm^{-1}): 3546, 3324 (NH), 1710 (C=O), 1613, 1546 (C=C), 1510, 1377 (N=O), 1357, 1185 (S=O).

N′-Nicotinylbenzenesulfonohydrazide **(4)**

$C_{12}H_{11}N_3O_3S$; White solid; Yield: 61%; R_f = 0.82 (*n*-hexane : ethyl acetate 7:3); m.p. 190-194 °C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.77 (s, 1H, H-2), 8.67 (m,1H, H-5), 8.06 (d, 1H, $J_{6,5}$= 8.0 Hz, H-6), 7.94 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 7.6 Hz, H-2′, H-6′), 7.90 (m, 1H, H-4′), 7.63 (m, 2H, H-3′, H-5′), 7.56 (m, 1H, H-4); ^{13}C-NMR (75 MHz, CD_3OD): δ_H 164.0, 148.3, 147.5, 136.2, 135.0, 131.3, 130.4, 129.0, 129.0, 127.2, 127.2, 125.2; EI-MS: (rel. abund.%), *m/z* 277 (4), 247 (5), 242 (4), 184 (3), 157 (2), 141 (12), 123 (13), 106 (100), 78(53), 51 (23); IR (KBr, cm^{-1}): 3523, 3206 (NH), 1697 (C=O), 1605, 1534 (C=C), 1365, 1175 (S=O).

5-Bromo-2-methoxy-N′-nicotinylbenzenesulfonohydrazide **(5)**

$C_{13}H_{12}BrN_3O_4S$; White solid; Yield: 67%; R_f = 0.85 (*n*-hexane : ethyl acetate 7:3); m.p. 228-230°C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.70 (s, 1H, H-2), 8.67 (d, 1H, $J_{6',4'}$ = 4.0Hz, H-6′), 8.06 (d, 1H, $J_{6,5}$= 8.0 Hz, H-6), 7.91 (m, 1H, H-3′), 7.71 (m, 1H, H-4′), 7.50 (m, 1H, H-5),7.14 (d, 1H, $J_{4,5}$= 8.8 Hz, H-4), 4.02 (s, 3H, 2′-OCH_3): EI-MS: (rel. abund.%), *m/z* 386 (12), 307 (20), 276 (2), 252 (18), 235 (41), 186 (26), 157 (18), 106 (40), 78 (76), 51 (36).

3,4-Dimethoxy-N′-nicotinylbenzenesulfono hydrazide **(6)**

$C_{14}H_{15}N_3O_5S$; White solid; Yield 68%; R_f = 0.88 (*n*-hexane : ethyl acetate 7:3); m.p. 233-235°C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.80 (s, 1H, H-2), 8.68 (d, 1H, $J_{2',5'}$ = 3.6 Hz, H-2′), 8.08 (d, 1H, $J_{6',5'}$ = 8.0 Hz, H-6′), 7.52 (m, 3H, H-4, H-5, H-6), 7.02 (d, 1H, $J_{5(4,6)}$= 8.4 Hz, H-5), 3.85 (s, 3H, 3′-OCH_3), 3.82 (s, 3H, 4′-OCH_3); ^{13}C-NMR (75 MHz, CD_3OD): δ_H 163.7, 153.2, 150.3, 148.5, 148.0, 135.2, 133.5, 130.4, 124.7, 117.9, 112.0, 115.4, 56.2, 56.3. EI-MS: (rel. abund.%), *m/z* 337 (45), 203 (35),185 (53), 153 (49), 106 (100), 78 (88), 51 (72): IR (KBr, cm^{-1}): 3535, 3319 (NH), 1688 (C=O), 1600, 1512 (C=C), 1342, 1165 (S=O), 1110, 1098 (C-O).

N-Nicotinoyl-2-nitrobenzenesulfonohydrazide **(7)**

$C_{12}H_{10}N_4O_5S$; White solid; Yield 67%; R_f = 0.81 (*n*-hexane : ethyl acetate 7:3); m.p. 254-256°C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.85 (s, 1H, H-2), 8.68 (m, 1H, H-3′), 8.15 (t, 2H, $J_{4'(3',5')/5'(6',4')}$ = 7.2 Hz, H-4′, H-5′), 7.89 (d, 1H, $J_{6,5}$= 8.0 Hz, H-6), 7,83(t, 1H, $J_{5(4,6)}$ = 7.2 Hz, H-5), 7.77 (m, 1H, H-6′), 7.52 (m, 1H, H-4):EI-MS(rel. abund.%), *m/z* 322 (3), 292 (2), 242 (2), 123 (100), 182 (3), 106 (39), 77 (85), 50 (40).

N-Nicotinoyl-4-propylbenzenesulfonohydrazide **(8)**

$C_{15}H_{17}N_3O_3S$; White solid; Yield 63%; R_f = 0.74 (*n*-hexane : ethyl acetate 7:3); m.p. 236-238°C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.76 (s, 1H, H-2), 8.66 (m, 1H, H-5), 8.05 (d, 1H, $J_{6,5}$= 7.6 Hz, H-6), 7.84 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.4 Hz, H-2′, H-6′), 7.50 (m, 1H, H-4), 7.33 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.0 Hz, H-3′, H-5′), 2.66 (t, 2H, $J_{CH2(CH2)}$ = 7.6 Hz, CH_2), 1.65 (m, 2H, CH_2), 0.90 (t, 3H, $J_{CH3(CH2)}$ = 7.2 Hz): EI-MS: (rel. abund.%) *m/z* 319 (50), 289 (10), 241 (51), 183 (26), 167 (31), 147 (9), 106 (100), 91 (42), 78 (63), 51 (19).

N-Nicotinoylmethanesulfonohydrazide **(9)**

$C_7H_9N_3O_3S$; White solid; Yield: 64%; R_f = 0.76 (*n*-hexane : ethyl acetate 7:3); m.p. 175-177°C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 9.00 (s, 1H, H-2), 8.73 (m, 1H, H-5), 8.28 (d, 1H, $J_{6,5}$= 8.0 Hz, H-6), 7.58 (m, 1H, H-4), 3.05 (s, 3H, CH_3):EI-MS: (rel. abund.%) *m/z* 215 (4), 185 (20), 136 (29), 106 (100), 78 (79), 65 (13), 51 (40).

2,4-Dimethoxy-N-nicotinoylbenzenesulfonohydrazide **(10)**

$C_{14}H_{15}N_3O_5S$; White solid; Yield: 64%; R_f = 0.63 (*n*-hexane : ethyl acetate 7:3); m.p. 234-236°C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 8.92 (s, 1H, H-2), 8.76 (m, 1H, H-6), 8.37 (m, 1H, H-3′), 8.21 (m, 1H, H-5), 7.95 (d, 2H, $J_{5',6'} = J_{6',5'}$ = 8.0 Hz, H-5′, H-6′), 7.54 (m, 1H, H-4), 3.85 (s, 3H, 2′-OCH_3), 3.63 (s, 3H, 4′-OCH_3): EI-MS: (rel. abund.%) *m/z* 337 (90), 307 (3), 274 (3), 243 (4), 229 (4), 201 (6), 185 (24),151 (69), 137 (17), 122 (26), 106 (48), 78 (41), 51 (24).

N-Nicotinoyloctane-1-sulfonohydrazide **(11)**

$C_{14}H_{23}N_3O_3S$; White solid; Yield: 51%; R_f = 0.65 (*n*-hexane : ethyl acetate 7:3); m.p. 192-194°C;

^{1}HNMR (400 MHz, CD_3OD) δ_H 8.98 (s, 1H, H-2), 8.72 (m, 1H, H-5), 8.26 (d, 1H, $J_{6,5}$= 8.0 Hz, H-6), 7.57 (m, 1H, H-4), 3.16 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 1.43 (m, 2H, CH_2), 1.32 (m, 4H, $(CH_2)_2$), 1.29 (m, 4H, $(CH_2)_2$), 0.90 (m, 3H, CH_3): EI-MS: (rel. abund.%) *m/z* 313 (2), 283 (2), 219 (7), 179 (9),161 (9), 121 (23), 106 (100), 57 (51).

2-Methyl-N′-nicotinoyl-5-nitrobenzenesulfonohydrazide **(12)**

$C_{13}H_{12}N_4O_5S$; White solid; Yield 71%; R_f = 0.59 (*n*-hexane : ethyl acetate 7:3); m.p. 260-262 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H 10.94 (s, 1H, NH), 8.77 (s, 1H, H-2), 8.69 (m, 1H, H-5), 8.54 (s, 1H, NH), 8.34 (m, 1H, H-6), 8.26 (m, 1H, H-6′), 7.98 (d, 1H, $J_{4',3'}$ = 8.0 Hz, H-4′), 7.71 (d, 1H, $J_{3',4'}$ = 8.4 Hz, H-3′), 7.47 (m, 1H, H-4): EI-MS: (rel. abund.%) *m/z* 319 (11), 336 (100), 289 (2), 257 (7), 224 (3), 205 (4), 167 (41), 121 (32), 106 (31), 78 (24), 63 (5).

3,5-Dichloro-2-hydroxy-N-nicotinoylbenzensulfonohyrazide **(13)**

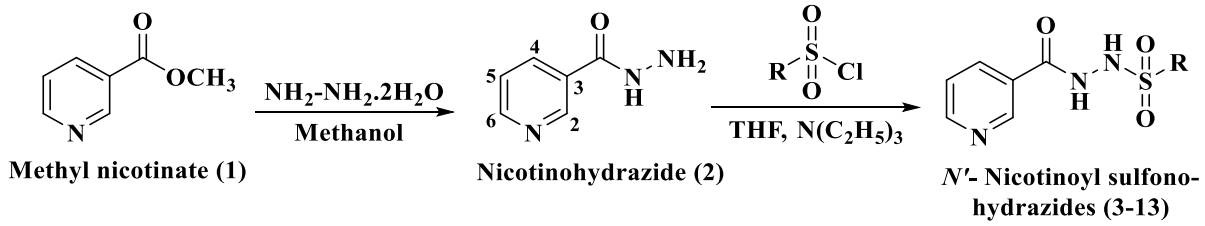
$C_{12}H_9Cl_2N_3O_4S$; White solid; Yield: 71%; R_f = 0.78 (*n*-hexane : ethyl acetate 7:3); m.p. 242-244°C; ^{1}H-NMR (400 MHz, CD_3OD) δ_H 11.10 (s, 1H, NH), 8.95 (s, 1H, H-2), 8.73 (m, 1H, H-5), 8.54 (s, 1H, NH), 8.15 (d, 1H, $J_{6,5}$= 8.0 Hz, H-6), 7.75 (m, 1H, NH), 7.54 (m, 2H, H-4′, H-6′), 7.41 (m, 1H, OH), 7.36 (m, 1H, H-4): EI-MS: (rel. abund.%) *m/z*361 (10), 283 (5), 267 (2), 224 (51), 194 (18), 162 (89), 136 (22), 123 (12), 106 (100), 78 (61), 63 (14), 51 (20).

Results and Discussion

Chemistry

First nicotinic hydrazide was synthesized by treating nicotinic acid methyl ester and hydrazine hydrate in methanol under reflux condition. Than, nicotinic hydrazide was treated with various sulfonyl chlorides in tetrahydrofuran (THF) in the presence of triethylamine base, to afford a variety of *N′*-nicotinoyl sulfonohydrazide (Table-1), which were crystalize from ethanol. Structures of synthetic compounds were determined by different spectroscopic techniques EI-MS, ^{1}H-, ^{13}C-NMR, and IR.

The substitution of sulfonyl group on nicotinic hydrazide was confirmed by disappearance of signal of amine group at δ 4.9 ppm from nicotinohydrazide and appearance of new signal at δ 11.48 ppm of conjugated –**NH-NH**-SO_2-R. In IR spectrum, characteristic absorption peak of primary amine disappeared and appeared in the range 3546-3206 cm^{-1} confirms the presence of –**NH-NH**-SO_2-R.



Scheme-1: Synthesis of *N′*-nicotinoylsulfonohydrazide derivatives **3-13**

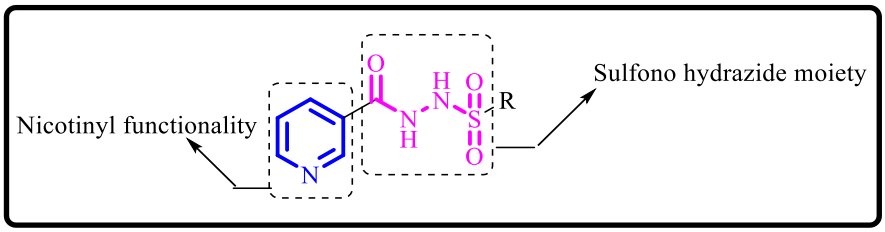
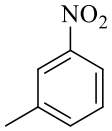
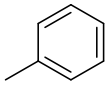
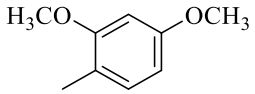
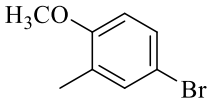
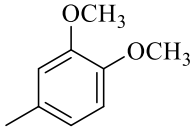
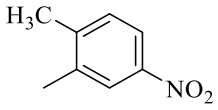
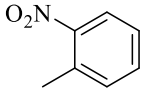
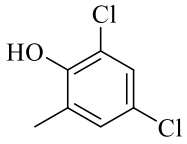
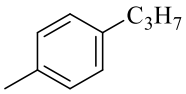


Fig. 2: General structure of synthetic compounds.

Table-1: Synthesis of *N'*-nicotinylsulfonohydrazide **(3-13).**

Compounds	R	Compounds	R
3		**9**	**-CH_3**
4		**10**	
5		**11**	**-C_8H_{17}**
6		**12**	
7		**13**	
8		-	-

Antibacterial activity

Synthetic compounds **3-13** were evaluated for antimicrbial activity against various strains of Gram positive bacteria such as *Bacillus cereus, Bacillus subtilis, Corynebacterium diphtheriae, Staphylococcus fecalis, Staphylococcus aureus,* and *MRSA* (*Methicillin-resistant Staphylococcus aureus*) and Gram negative bacteria including *Klebsiella pneumoniae, Escherichia coli, Salmonella Para typhi B, Salmonella tyhpi,* and *Pseudomonas aeruginosa* (Table-2).

Antibacterial potential was measured on the basis of zone of inhibition growth. Synthesized compounds exhibited antibacterial potential in the range of (ZOI = 6-20 mm) when screened against variety of Gram-positive and Gram-negative bacterial strains. Almost all the derivatives **3-13** were showed moderate zone of inhibition against *Escherichia coli*, MRSA, *Bacillus subtilis,* and *Staphylococcus fecalis.* Especially, compound **13** having hydroxy at *ortho* and two chloro substitutions at *meta* positions showed outstanding antibacterial activity in case of *Staphylococcus fecalis* (ZOI = 20 mm). Whereas, other derivatives showed weak zone of inhibition against *Staphylococcus fecalis* (ZOI = 7-10 mm). It is interesting to note that other derivatives do not possess chloro or hydroxy substitution which means that these groups play an important role in the active nature of compounds. Compound **13** also showed moderate activities against *Escherichia coli* (ZOI = 10 mm), *Salmonella paratyphi B* (ZOI = 13 mm), and *MRSA* (ZOI = 11 mm). Other compounds **4**, **5**, **7**, **9**, and **11-13** also showed mild susceptibility against MRSA (ZOI = 8-12 mm) (Fig-3). Compound **4** with no substitution at benzene ring showed moderate inhibition against *MRSA* (ZOI = 11 mm) and weak inhibition against *Escherichia coli, Klebsiella pneumoniae, Salmonella paratyphi B, Bacillus cereus, Bacillus subtilis,* and *Staphylococcus fecalis*. Amongst nitro substituted derivatives **3** and **7**, analog **7** having nitro group at *ortho* position showed respectively, showed moderate inhibition against *Salmonella paratyphi B* (ZOI = 13 mm) and *Bacillus cereus* (ZOI = 11 mm). However, compound **3** with nitro substitution at meta position showed weak inhibition against *Escherichia coli* and *Bacillus cereus*, which showed that position of nitro group is playing important role in the activity. Further more, compound **11** having *n*-octyl substitution showed moderate inhibtion against *MRSA* (ZOI = 12 mm) (Table-2).

Table-2: *In vitro* antibacterial activity of Gram positive and Gram negative bacteria (zone of inhibition in mm).

Bacterial Strains	Compounds											Standard
	3	4	5	6	7	8	9	10	11	12	13	
Gram negative bacteria												Gentamicin
Escherichia coli	8	6	6	9	9	8	12	9	-	8	10	29
Klebsiella pneumoniae	-	9	-	6	9	-	8	6	-	9	-	25
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	-	25
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	22
Salmonella paratyphi B	-	8	10	-	13	7	-	-	9	10	13	25
Gram positive bacteria												
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	25
MRSA	-	11	9	-	10	-	8	-	12	9	11	20
Bacillus cereus	8	9	-	-	11	9	9	-	8	8	7	25
Bacillus subtilis	-	8	9	9	8	10	9	9	-	10	6	22
Staphylococcus fecalis	-	9	10	9	8	8	7	9	7	7	20	25

Keys: - Zone of inhibition(ZOI) Key: Weakly active ≥ 8-10mm, Moderately active ≥ 12-14mm, Good activity(susceptible) ≥ 15

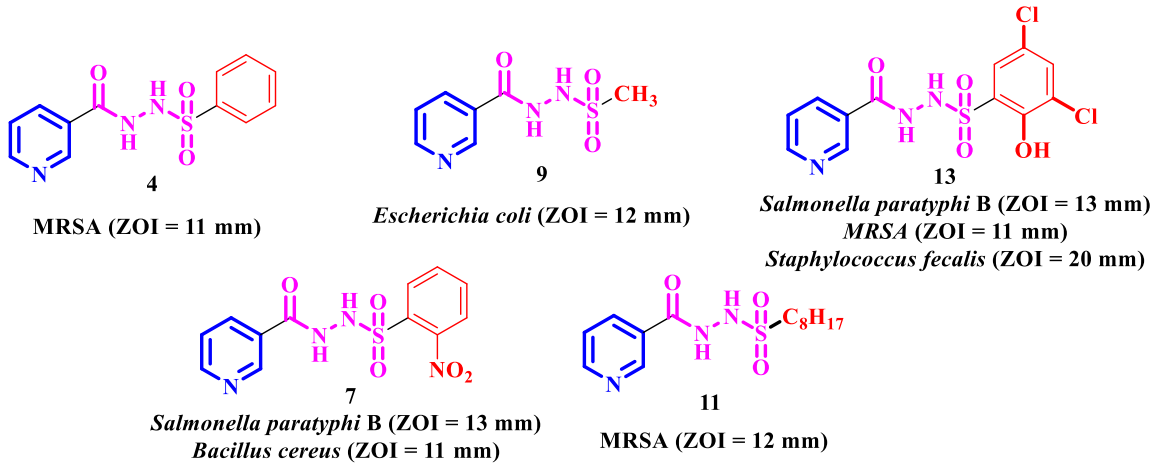


Fig. 3: Compounds **4**, **7**, **9**, **11**, and **13** with Good to Moderate Antibacterial Potential.

Table-3: *In vitro* antifungal activity (Zone of inhibition in mm using the disc diffusion method).

Fungal Strains	Compounds											Standard
	3	4	5	6	7	8	9	10	11	12	13	Ketoconazole
Saccharomyces cerevisiae	-	-	-	-	-	-	-	-	-	-	-	22
Microsporum canin	-	-	-	-	-	-	-	-	-	-	10	24
Rhizopus	-	-	-	-	-	-	-	-	-	-	20	22
Aspergillus niger	-	-	-	-	-	-	10	-	-	-	9	24
Candida albicans	-	-	-	-	-	-	-	-	-	-	-	22
Candida tropicalis	-	-	-	-	-	-	-	-	-	10	9	22

Zone of inhibition (ZOI) Key: Weakly active ≥ 8-10mm, Moderately active ≥ 12-14mm, Good activity(susceptible) ≥ 15

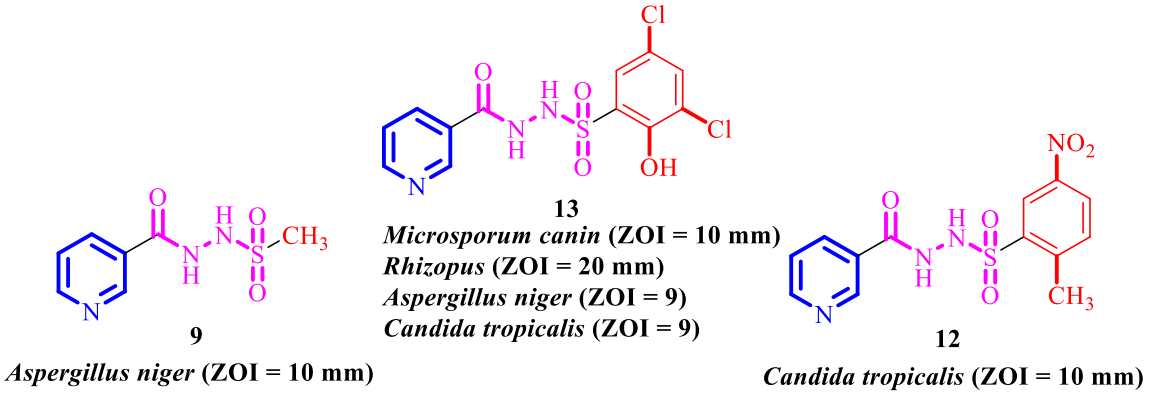


Fig. 4: Compounds **9**, **12**, and **13** with Good to Moderate Antifungal Potential.

Antifungal activity

Synthetic compounds **3-13** were also evaluated for antifungal activities against different fungal strains including *Saccharomyces cerevisiae*, *Microsporum canin*, *Rhizopus*, *Aspergillus niger*, *Candida albicans*, and *Candida tropicalis*. Antifungal potential was measured for all synthetic derivatives in terms of zone of inhibition growth in mm. Ketoconazole was uses as positive control (standard). It is interesting to note that compound **13** with hydroxy and chloro substitutions, also showed excellent inhibition in case of *Rhizopus sp.* (ZOI= 20 mm) and moderate inhibition against *Candida tropicalis*, *Aspergillus niger*, and *Microsporum canis*. Other compounds showed comparitively weak zone of inhibition. Compound **9** having only methyl substitution revealed a weak zone of inhibition growth in case of *Aspergillus niger* whereas derivative **12** with nitro and methyl groups showed weak zone of inhibition growth against *Candida tropicalis* (Fig-4).

Conclusion

Synthetic *N′*-nicotinoylsulfonohydrazide derivatives **3-13** were evaluated for their antimicrobial inhibitory potential against various bacterial strains. Compounds showed moderate to weak antibacterial activity. Interestingly, compound **13** having hydroxy and chloro substitutions were found to be prominent among all derivatives. All derivatives were also evaluated for antifungal activity. Only compound **13** also have good antifungal activity against *Rhizopus.*

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