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Synthesis and Investigation of Antimicrobial Activity of Some Nifuroxazide Analogues

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A series of nifuroxazide analogues [(**2a-2e**)-(**10a-10f**)] have been synthesized, structurally identified and tested for antimicrobial activity against a panel of bacteria (Gram-positive and Gram-negative) and the yeast-like pathogenic fungus *Candida albicans*. The most active compound in this series was 4-amino-benzoic acid (5-nitro-furan-2-ylmethylene)-hydrazide (**2b**) and 2-amino-benzoic acid (5-nitro-furan-2-ylmethylene)-hydrazide (**2d**). Furthermore, compounds (**9a-9g**) and (**10a-10g**) recorded no activity against selected species except compounds (**9f**) and (**10f**) suggesting that using furoic hydrazide and the corresponding hydrazide of thiophene did not improve the antimicrobial activities for this type of compounds. Regarding the activity against *Candida albicans*, all compounds showed no activity with an exception of substituted nitro furan (**2a-2d**).

Keywords: Nifuroxazide, Hydrazide-hydrazone, Antimicrobial activity.

INTRODUCTION

Development of antimicrobial agents to treat infections has been one of the most notable medical achievements of the past century[1]. As well as the development of resistance of bacteria against these antimicrobial agents has become a widespread medical concern, especially with the appearance of nosocomial pathogens[1-3]. Because of this critical situation, much effort has been directed to design new drugs by molecular modification, which considers a quite promising strategy in the design and development of drug analogues[4,5].

Nifuroxazide (NXZ, Fig. 1) is one of the synthetic antimicrobial agents which has wide spectrum of activity[2-11]. It was demonstrated that the pharmacological activity is mainly due to its configuration and is greatly diminished if the nitro group is shifted from position 5 to either position 4 or 3[9]. The antimicrobial activities of nifuroxazide are related to tow major antimicrobial classes nitrofurans class and hydrazide-hydrazone class Fig. 1.

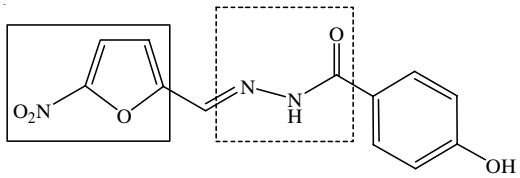


Fig. 1. Nifuroxazide classification

Nitrofurans are a class of drugs typically used as antibiotics or antimicrobials[12]. Many drugs of interest for bacteriology have been found in this broad class of compounds. As will as hydrazone group that is an important group that possess a recognized antimicrobial activity. Furthermore, a number of hydrazide-hydrazones structure have been demonstrated to possess interesting as antimicrobial[1,3], antituberculosis[5,7], antiparasitic[13], anti-inflammatory[14], anti-tumour[15], anti-HIV[16], inhibitor of anthrax lethal factor[17], anticonvulsant[18], analgesic and antiplatelet activities[19].

This study was aimed to synthesize, structurally identified and tested for antimicrobial activity of series of nifuroxazide analogues (Fig. 2) against two Gram-positive bacteria [*Staphylococcus aureus* (ATCC 25923); *Bacillus subtilis* (ATCC 6633)]; three Gram-negative bacteria [*EC*: *Escherichia coli* (ATCC 25922); *ST*: *Salmonella susi* (ATTC 13070); *PA*: *Pseudomonas aeruginosa* (ATCC27853)] and one Fungus [*Candida albicans* (ATCC 10231)].

EXPERIMENTAL

Melting points (°C, uncorrected) were determined using an electrothermal's IA9000 series digital capillary melting point apparatus. IR spectra (KBr disks) were recorded on a Perkin Elmer FT spectrophotometer 1000. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECP 400 NMR spectrometer operating at 400 MHz using DMSO-d_6 as solvents with TMS

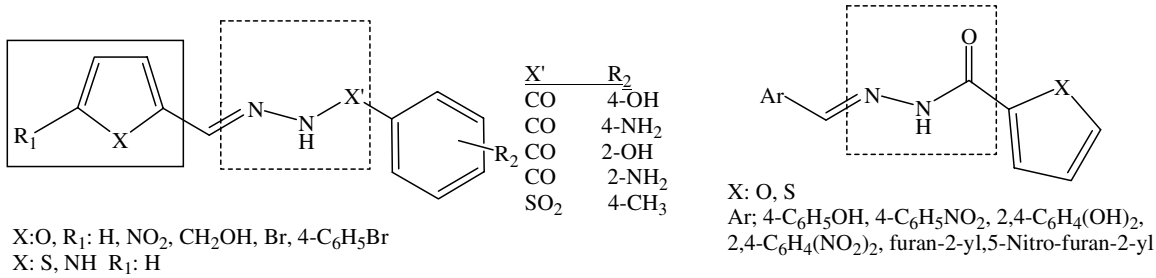


Fig. 2. Molecular modification of nifuroxazide

as internal standard, Chemical shifts are given in ppm (δ-scale) and the coupling constants are given in Hertz. Monitoring the reactions and checking the purity of the final product were carried out by thin layer chromatography using TLC pre-coated silica gel sheets (60 F254, Merck) and ethanol-chloroform (1:9) as eluent. The spots were detected by exposure to UV-lamp at λ 254 nm for few seconds.

Synthesis: Substituted benzhydrazides (**1a-1d**) were synthesized from respective methyl substituted benzoate (1 mmol) with hydrazine hydrate 99 % (30 mmol) under reflux for 3-4 h. The solid product obtained after cooling was filtered off and dried[2]. Benzhydrazides (**1a**) and *p*-toluenesulfonyl hydrazide (**1e**) have been synthesized by adding (10 mmol) of 50 % hydrazine hydrate with benzoyl chloride (1 mmol) and *p*-toluenesulfonyl chloride (1 mmol) respectively under reflux for 0.5 h. The solid products were collected after cooling and dried[1].

4-Hydroxy-benzoic acid hydrazide (1a): White powder; m.p. 114 °C; IR (cm^{-1}): 3275-3296 ν(N–H and NH_2); 1669 ν(C=O); ^{1}H NMR (DMSO-d_6): 10.70 (1H, s, OH); 9.08 (1H, s, NH); 7.71 (d, *J* = 8.79, 2H, H2 and H6); 6.89 (d, *J* = 8.79, 2H, H3 and H5); 4.78 (2H, s, NH_2).

4-Amino-benzoic acid hydrazide (1b): White powder; m.p. 225 °C; IR (cm^{-1}): 3233-3300 ν(N–H and NH_2); 1660 ν(C=O); ^{1}H NMR (DMSO-d_6): 9.15 (1H, s, NH); 7.51 (d, *J* = 8.79, 2H, H2 and H6); 6.56 (d, *J* = 8.79, 2H, H3 and H5); 5.43 (s, 2H, NH_2); 4.04 (2H, s, NH_2).

2-Hydroxy-benzoic acid hydrazide (1c): White powder; m.p. 149 °C; IR (cm^{-1}): 3200-3300 ν(N–H and NH_2); 1660 ν(C=O); ^{1}H NMR (DMSO-d_6): 12.47 (s, 1H, OH); 10.04 (1H, s, NH); 7.77 (d, 1H, H6); 7.36 (t, 1H, H4); 6.86-6.84 (m, 2H, H5 and H3); 4.62 (2H, s, NH_2).

2-Amino-benzoic acid hydrazide (1d): Beige powder; Yield: 87.61 %; IR (cm^{-1}): 3269-3319 ν(N–H and NH_2); 1645 ν(C=O); ^{1}H NMR (DMSO-d_6): 9.46 (1H, s, NH); 7.41 (d.d, *J* = 8.05, *J* = 1.44, 1H, H5); 7.10 (t.d, *J* = 8.25, *J* = 1.47, 1H, H4); 6.68 (d.d, *J* = 8.80, *J* = 1.11, 1H, H6); 6.46 (t.d, *J* = 7.87, *J* = 1.11, 1H, H3); 6.32 (s, 2H, NH_2); 4.36 (2H, s, NH_2).

***p*-Toluenesulfonyl hydrazide (1e):** White powder; m.p. 106-108 °C; IR (cm^{-1}): 3389-3260 ν(N–H and NH_2); ^{1}H NMR (DMSO-d_6): 8.32 (1H, s, NH); 7.72 (d, *J* = 8.79, 2H, H2 and H6); 7.39 (d, *J* = 8.79, 2H, H3 and H5); 4.01 (s, 2H, NH_2); 2.29 (3H, s, CH_3).

Synthesis: The target compounds (**2a-2e**)-(**6a-6f**) were synthesized by reacting equimolar proportion of substituted benzhydrazides and benzenesulfonyl hydrazide with 5-substituted 2-furan carboxaldehyde in ethanol absolute under reflux[2] for 2 h. Compounds [(**7a-7e**)-(**8b-8e**)] were synthesized by replacing the furan ring with thiophene and pyrrole. The compounds [(**9a-9f**)- (**10a-10f**)] were also prepared from the reaction of furoic hydrazide and the corresponding hydrazide of thiophene with a variety of aromatic aldehydes, furan-2-carboxaldehyde and thiophene-2-carboxaldehyde in ethanol absolute ethanol under reflux for 2 h. The progress of the reaction was monitor by TLC. The insoluble product was filtered off.

4-Hydroxy-benzoic acid 5-nitro-furan-2-ylmethylene-hydrazide (2a): Yellow powder; Yield 91.40 %; m.p. 280-282 °C; IR 3365 ν(O–H); 3245 ν(N–H); 1674 ν(C=O); ^{1}H NMR (DMSO-d_6): 10.04 (s, 1H, NH); 10.24 (s, 1H, OH); 8.37 (s, 1H, CH=N); 7.82 (d, *J* = 8.08.2 H, H2' and H6'); 7.77 (d, *J* = 3.64, 1H, H4); 7.22 (d, *J* = 4.4, 1H, H3); 6.88 (d, *J* = 8.8, 2H, H3' and H5'); ^{13}C NMR: 163.20 (CO); 161.66 (C-4'); 152.61 (C-5); 152.35 (C-2); 134 (CH); 130.52 (C-2' and C-6'); 123.71 (C-1'); 115.70 (C-3' and C-5'); 115.38 (C-4); 115.27 (C-3); MS: $C_{12}H_9N_3O_5^{+\cdot}$, M^+ 275 (5.00 %); M+1 276.27 (100 %); $C_{12}H_{10}N_2O_3^{+\cdot}$, *m/z* 130.27 (9 %); $C_7H_7NO_2^+$, *m/z* 137.18 (70 %); $C_7H_5O_2^+$, *m/z* 121.35 (20 %); $C_5H_5N_3O_3^+$, *m/z* 155.27 (37 %); $C_5H_2N_2O_3^+$, *m/z* 138 (12 %).

4-Amino-benzoic acid 5-nitro-furan-2-ylmethylene-hydrazide (2b): Red powder; Yield 92.42 %; m.p. >300 °C; IR (cm^{-1}): 3452-3313 ν(N–H); 1664 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.89 (s, 1H, NH); 8.34 (s, 1H, CH=N); 7.76 (d, *J* = 3.64, 1H, H4); 7.69 (d, *J* = 8.56.2H, H2' and H6'); 7.18 (d, *J* = 3.68, 1H, H3); 6.62 (d, *J* = 8.56, 2H, H3' and H5'); 5.89 (s, 2H, NH_2); ^{13}C NMR: 163.91 (CO); 153.32 (C-5); 152.90 (C-2); 152.25 (C-4'); 134.07 (CH); 130.27 (C-2' and C-6'); 119.15 (C-1'); 115.38 (C-4); 114.92 (C-3); 113.22 (C-3' and C-5').

2-Hydroxy-benzoic acid 5-nitro-furan-2-yl-methylene-hydrazide (2c): Yellow fine scales; Yield 88.56 %; m.p. 257-259 °C; IR (cm^{-1}): 3465 ν(O–H); 3227 ν(N–H); 1630 ν(C=O); ^{1}H NMR (DMSO-d_6): 12.11 (s, 1H.NH); 11.52 (s, 1H, OH); 8.42 (s, 1H, CH=N); 7.84 (d, *J* = 8.08, 1H, H6'); 7.81 (d, *J* = 3.64, 1H, H4); 7.45 (t, *J* = 8.04, 1H, H4'); 7.28 (d, *J* = 3.68, 1H, H3); 6.98 (t, *J* = 8.08, 1H, H5'); 6.97 (d, *J* = 7.36, 1H, H3'); ^{13}C NMR: 165.19 (CO); 158.86 (C-2'); 152.53 (C-5); 152.15 (C-2); 136.65 (CH); 134.55 (C-4'); 129.64 (C-6'); 119.72 (C-5'); 117, 73 (C-1'); 117.08 (C-4); 116.16 (C-3); 115.18 (C-3').

2-Amino-benzoic acid 5-nitro-furan-2-yl-methylene-hydrazide (2d): Yellow powder; Yield 93.55 %; m.p. 213-214 °C; IR (cm^{-1}): 3395, 3306-3234 ν(N–H); 1659 ν(C=O); ^{1}H NMR (DMSO-d_6): 12.01 (s, 1H.NH); 8.33 (s, 1H, CH=N); 7.79 (d, *J* = 404, 1H, H4); 7.85 (d, *J* = 8.08, 1H, H6'); 7.23-7.21 (m, 2H, H4' and H3); 6.78 (d, *J* = 8.04, 1H, H3'); 6.58 (t, *J* = 7.32, 1H, H5'); 6.48 (s, 2H, NH_2); ^{13}C NMR: 166.08 (CO); 152.65 (C-5); 152.35 (C-2); 150.98 (C-2'); 134.87 (CH);

133.36 (C-4'); 128.96 (C-6'); 117.10 (C-5'); 115.31 (C-4 and C-3'); 115.18 (C-1'); 113.01 (C-3).

4-Methyl-benzenesulfonyl (5-nitro furan-2-yl-methylene)hydrazide (2e): Yellow plates; Yield 55.94 %; m.p. 130-131 °C; IR (cm^{-1}): 3180 ν(N–H); 1355-1158 ν($(S=O)_2$); ^{1}H NMR (DMSO-d_6): 12.14 (s, 1H, NH); 7.86 (s, 1H, CH=N); 7.76 (d, *J* = 8.04, 2H, H2' and H6'); 7.71 (d, *J* = 3.68, 1H, H4); 7.43 (d, *J* = 8.08, 2H, H3' and H5'); 7.13 (d, *J* = 3.68, 1H, H3); 2.37 (s, 3H, CH_3); ^{13}C NMR: 152.37 (C-5); 151.27 (C-2); 144.45 (C-4'); 136.44 (C-1'); 135.21 (CH); 130.45 (C-3' and C-5'); 127.68 (C-2' and C-6'); 115.90 (C-4); 114.99 (C-3); 21 (CH_3).

4-Hydroxy-benzoic acid furan-2-yl-methylene-hydrazide (3a): Yellowish white plates; Yield 73.39 %; m.p. 221-223 °C; IR (cm^{-1}): 3460 ν(O–H); 3254 ν(N–H); 1617 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.59 (1H, s, NH); 10.11 (s, 1H, OH); 8.32 (1H, s, CH=N); 7.83 (s, 1H, H5); 7.79 (d, *J* = 8.08, 2H, H2' and H6'); 6.89 (d, *J* = 2.2, 1H, H3); 6.87 (d, *J* = 8.8, 2H, H3' and H5'); 6.63 (t, *J* = 2.2, 1H, H4); ^{13}C NMR:163.23 (CO); 161.25 (C-4'); 150.17 (C-2); 145.51 (C-5); 137.24 (CH); 130.19 (C-3' and C-5'); 124.51 (C-1'); 115.58 (C-3' and C-5'); 113.54 (C-3); 112.71 (C-4).

4-Amino-benzoic acid furan-2-ylmethylene-hydrazide (3b): Beige powder; Yield 75.76 %; m.p. 190-191 °C; IR (cm^{-1}): 3232-3390-3300 ν(N–H); 1638 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.43 (1H, s, NH); 8.30 (1H, s, CH=N); 7.81 (s, 1H, H5); 7.67 (d, *J* = 8.8, 2H, H2' and H6'); 6.85 (d, *J* = 3.68, 1H, H3); 6.61 (3, *J* = 3.64, 1H, H4); 6.60 (d, *J* = 8.8, 2H, H3' and H5'); 5.80 (s, 2H, NH_2); ^{13}C NMR:163.65 (CO); 152.87 (C-4'); 150.38 (C-2); 145.28 (C-5); 136.43 (CH); 129.92 (C-2' and C-6'); 119.94 (C-1'); 113.17 (C-3' and C-5'); 113.02 (C-3); 112.66 (C-4).

2-Hydroxy-benzoic acid furan-2-yl-methylene-hydrazide (3c): Beige powder; Yield 77.55 %; m.p. 222-224 °C; IR (cm^{-1}): 3470 ν(O–H); 3247 ν(N–H); 1635 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.83 (1H, s, NH); 11.80 (s, 1H, OH); 8.35 (1H, s, CH=N); 7.87 (d, *J* = 8.04, 1H, H6'); 7.88 (s, 1H, H5); 7.66 (d, 1H, H3); 7.44 (t, *J* = 8.08, 1H, H4'); 6.97 (m, 2H, H3', H5' and H4); ^{13}C NMR:165.29 (CO); 159.55 (C-2'); 149.79 (C-2); 146.02 (C-5); 138.93 (CH); 134.38 (C-4'); 129.02 (C-6'); 119.50 (C-5'); 117.82 (C-1'); 116.45 (C-3'); 114.70 (C-3); 112.84 (C-4).

4-Methyl-benzenesulfonyl (furan-2-yl-methylene)-hydrazide (3e): Yellowish brown cubes; Yield 84.16 %; m.p. 110-112 °C; IR (cm^{-1}): 3154 ν(N–H); 1316-1168 ν($(S=O)_2$); 1350 ν(CH_3); ^{1}H NMR (DMSO-d_6): 11.44 (1H, s, NH); 7.78 (1H, s, CH=N); 7.75 (d, *J* = 1.48, 1H, H5); 7.73 (d, *J* = 8.08, 2H, H2' and H6'); 7.40 (d, *J* = 8.08, 2H, H3' and H5'); 6, 80 (d, *J* = 2.92, 1H, H3); 6.55 (d.d, *J* = 3.28, *J* = 2.2, 1H, H4); 2.35 (s, 3H, CH_3); 13C NMR: 147.90 (C-2); 144.38 (C-5); 142.83 (C-4'); 136.27 (C-1'); 135.43 (CH); 129.03 (C-3'and C-5'); 126.51 (C-2'and C-6'); 113.27 (C-3); 111.34 (C-4); 20.31 (CH_3).

4-Hydroxy-benzoic acid 5-hydroxymethyl-furan-2-ylmethylene-hydrazide (4a): Yellowish white plate; Yield 86.15 %; m.p. 202-203 °C; IR (cm^{-1}): 3400 ν(O–H); 3257 ν(N–H); 1630 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.57 (1H, s, NH); 10.14 (1H, s, OH); 8.25 (1H, s, N=CH); 7.79 (d, *J* = 8.8, 2H, H2' and H6'); 6.86 (d, *J* = 8.084, 2H, H3' and H5'); 6.82 (d, *J* = 2.2, 1H, H3); 6.44 (d, *J* = 2.96, 1H, H4); 5.42 (s, 1H, OH); 4.45 (s, 2H, CH_2). ^{13}C NMR: 163.20 (CO); 161.23 (C-4'); 158.17 (C-5); 149.36 (C-2); 137.08 (C=NH); 130.19 (C-2' and C-6'); 124.35 (C-1'); 115.57 (C-3' and C-5'); 114.74 (C-3); 109.72 (C-4); 56.28 (CH_2).

4-Amino-benzoic acid 5-hydroxymethyl-furan-2-ylmethylene-hydrazide (4b): Brown powder; Yield 95.79 %; m.p.182-184 °C; IR (cm^{-1}): 3261 ν(N–H); 1685 ν(C=O), ^{1}H NMR (DMSO-d_6): 11.84 (1H, s, NH); 8.23 (1H, s, CH); 7.65 (d, *J* = 8.8, 2H, H2' and H6'); 6.78 (d, *J* = 2.92, 1H, H3); 6.59 (d, *J* = 8.8, 2H, H3' and H5'); 6.44 (d, *J* = 2.92, 1H, H4); 5.79 (s, 2H, NH_2); 5.42 (d, *J* = 5.84 1H, OH); 4.45 (d, *J* = 5.12 2H, CH_2); ^{13}C NMR: 157.94 (C-5); 157.73 (CO); 152.84 (C-4'); 149.55 (C-2); 136.23 (CH=N); 129.88 (C-2' and C-6'); 119.95 (C-1'); 114.21 (C-3); 113.14 (C-3' and C-5'); 109.68 (C-4); 56.27 (CH_2).

2-Hydroxy-benzoic acid 5-hydroxymethyl-furan-2-ylmethylene-hydrazide (4c): Yellowish white plate; Yield 93.61 %; m.p. 171-172 °C; IR (cm^{-1}): 3470 ν(O–H); 3275 ν(N–H); 1630 ν(C=O); ^{1}H NMR (DMSO-d_6): 12.20 (s, 1H, OH); 11.77 (1H, s, NH); 8.30 (1H, s, CH); 7.56 (d, *J* = 8.08, 1H, H6'); 7.44 (t, *J* = 8.8, 1H, H4'); 6.99-6.93 (m, 2H, H3' and H5'). 6.89 (d, *J* = 2.92, 1H, H3); 6.47 (d, *J* = 2.92, 1H, H4); 4.59 (s, 1H, OH); 4.47 (s, 2H, CH_2); ^{13}C NMR: 165.26 (CO); 159.59 (C-2'); 158.71 (C-5); 148.96 (C-2); 138.81 (CH=N); 134.37 (C-4'); 129.60 (C-6'); 119.47 (C-5'); 117.83 (C-1'); 116.41 (C-3'); 115.94 (C-3); 109.82 (C-4); 56.52 (CH_2).

2-Amino-benzoic acid 5-hydroxymethyl-furan-2-ylmethylene-hydrazide (4d): Yellowish plate; Yield 95.94 %; m.p.177-178 °C; IR (cm^{-1}): 3200 ν(N–H); 1620 ν(C=O), ^{1}H NMR (DMSO-d_6): 11.53 (1H, s, NH); 8.22 (1H, s, CH); 7.53 (d, *J* = 8.04, 1H, H6'); 7.19 (t, *J* = 8.08, 1H, H4'); 6.82 (d, *J* = 2.96, 1H, H3); 6.76 (d, *J* = 8.08, 1H, H3'); 6.56 (t, *J* = 8.05, 1H, H5'); 6.44 (d, *J* = 2.92, 1H, H4); 6.37 (s, 2H, NH_2); 5.42 (s, 1H, OH); 4.45 (d, *J* = 5.12, 2H, CH_2); ^{13}C NMR: 162.80 (CO); 158.18 (C-5); 150.56 (C-2'); 149.38 (C-2); 137.07 (CH=N); 132.82 (C-4'); 128.81 (C-6'); 116.92 (C-5'); 115.18 (C-3'); 114.66 (C-3); 113.32 (C-1'); 109.73 (C-4); 56.28 (CH_2).

4-Hydroxy-benzoic acid 5-bromo-furan-2-ylmethylene-hydrazide (5a): Beige powder; Yield 94.26 %; m.p.175-176 °C; IR (cm^{-1}): 3484 ν(O–H); 3247 ν(N–H); 1635 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.66 (s, 1H, NH); 10.15 (s, 1H, OH); 8.23 (s, 1H, CH=N); 7.79 (d, *J* = 8.8, 2H, $H_{2'}$ and $H_{6'}$); 6.94 (d, *J* = 3.68, 1H, H_4); 6.87 (d, *J* = 8.8, 2H, $H_{3'}$ and $H_{5'}$); 6.75 (d, *J* = 3.68, 1H, H_3); ^{13}C NMR: 163.29 (CO); 161.31 (C-4'); 152.12 (C-2); 135.97 (CH); 130.25 (C-2' andC-6'); 124.85 (C-5); 124.20 (C-1'); 116.26 (C-4); 115.74 (C-3' and C-5'); 114.73 (C-3).

4-Amino-benzoic acid 5-bromo-furan-2-ylmethylene-hydrazide (5b): Orange fine needles; Yield 95.34 %; m.p. > 300 °C; IR (cm^{-1}): 3225 ν(N–H); 1620 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.49 (1H, s, NH); 8.21 (1H, s, CH=N); 7.66 (d, *J* = 8.56, 2H, H2' and H6'); 6.90 (d, *J* = 3.68, 1H, H4); 6.74 (d, *J* = 3.68, 1H, H3); 6.61 (d, *J* = 8.56, 2H, H3' and H5'); 5.81 (s, 2H, NH_2); ^{13}C NMR: 163.55 (CO); 152.96 (C-4'); 152.32 (C-2); 135.13 (CH); 129.96 (C-2' and C-6'); 124.54 (C-5); 119.76 (C-1'); 115.74 (C-4); 114.66 (C-3); 113.16 (C-3' and C-5').

2-Hydroxy-benzoic acid 5-bromo-furan-2-ylmethylene-hydrazide (5c): Beige powder; Yield 89.32 %; m.p.219-221 °C; IR (cm^{-1}): 3480 ν(O–H); 3245 ν(N–H); 1624 ν(C=O). ^{1}H NMR (DMSO-d_6): 11.76 (s, 1H, OH); 11.84 (1H, s, NH); 8.27 (1H, s, CH=N); 7.85 (d, *J* = 7.32, 1H, H6'); 7.43 (t, *J* = 7.36, 1H, H4'); 6.99 (m, 3H, H3, H3' and H5'); 6.78 (d, 1H, H_4); ^{13}C NMR: 165.28 (CO); 159.41 (C-2'); 151.74 (C-2); 137.64 (CH); 134.41 (C-4'); 129.13 (C-6'); 125.48 (C-5); 119.54 (C-5'); 117.80 (C-4); 117.29 (C-3'); 116.51 (C-1'); 114.86 (C-3).

2-Amino-benzoic acid 5-bromo-furan-2-ylmethylene-hydrazide (5d): Orange powder; Yield 76.25 %; m.p.133-134 °C; IR (cm^{-1}): 3241 ν(N–H); 1653 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.64 (1H, s, NH); 8.20 (1H, s, CH=N); 7.55 (d, *J* = 8.08, 1H, H6'); 7.20 (t, *J* = 8.08, 1H, H4'); 6.93 (d, *J* = 3.68, 1H, H4); 6.77 (d, *J* = 9.52, 1H, H3'); 6.74 (d, *J* = 3.68, 1H, H3); 6.57 (t, *J* = 7.32, 1H, H5'); 6.41 (s, 2H, NH_2); ^{13}C NMR: 165.91 (CO); 152.15 (C-2); 150.64 (C-2'); 135.94 (CH); 132.95 (C-4'); 128.85 (C-6'); 124.83 (C-5); 116.99 (C-4); 116.20 (C-5'); 115.21 (C-3'); 114.73 (C-3); 113.75 (C-1').

4-Methyl-benzenesulfonyl (5-bromo furan-2-ylmethylene)-hydrazide (5e): Beige powder; Yield 20.27 %; m.p.116-118 °C; IR (cm^{-1}): 3175 ν(N–H); 1342-1185 ν(S (=O)$_2$). ^{1}H NMR (DMSO-d_6): 11.55 (1H, s, NH); 7.73 (d, *J* = 8.8, 2H, H2' and H6'); 7.70 (1H, s, CH=N); 7.42 (d, *J* = 7.32, 2H, H3' and H5'); 6.85 (d, *J* = 3.64, 1H, H4); 6.69 (d, *J* = 2.92, 1H, H3); 2.37 (s, 3H, CH_3); ^{13}C NMR: 151.09 (C-2); 144.13 (C-4'); 136.64 (C-1'); 136.33 (CH=N); 130.33 (C-3' and C-5'); 127.67 (C-2' and C-6'); 125.12 (C-5); 116.94 (C-4); 114.68 (C-3); 21.57 (CH_3).

4-Hydroxy-benzoic acid 5-[4-bromophenyl]-furan-2-ylmethylene-hydrazide (6a): Beige plates; Yield 81.29 %; m.p. 162-164 °C; IR (cm^{-1}): 3412 ν(O–H); 3231 ν(N–H); 1615 ν(C=O); 1504 and 1469 ν(C=C). ^{1}H NMR (DMSO-d_6): 11.67 (s, 1H, NH); 10.15 (s, 1H, OH); 8.36 (s, 1H, CH=N); 7.81 (d, *J* = 8.04, 2H, H2' and H6'); 7.75 (d, *J* = 8.04, 2H, H3" and H5"); 7.67 (d, *J* = 8.04, 2H, H2" and H4"); 6.20 (d, *J* = 3.68, 1H, H4); 7.03 (d, *J* = 3.68, 1H, H3); 6.88 (d, *J* = 8.8, 2H, H3' and H5'); ^{13}C NMR: 163.21 (CO); 161.28 (C-4'); 153.97 (C-5); 150.19 (C-2); 136.92 (CH=N); 132.56 (C-3" and C-5"); 130.22 (C-2' andC-6'); 129.29 (C-1"); 126.39 (C-2" and C-6"); 124.32 (C-1'); 121.76 (C-4"); 116.21 (C-4); 115.60 (C-3' and C-5'); 109.67 (C-3).

4-Amino-benzoic acid 5-[4-bromophenyl]-furan-2-ylmethylene-hydrazide (6b): Yellowish white plates; Yield 77.03 %; m.p. 206-208 °C; IR (cm^{-1}): 3219 ν(N–H); 1612 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.52 (1H, s, NH); 8.35 (1H, s, CH=N); 7.72 (d, *J* = 8.52, 2H, H3" and H5"); 7.69 (d, *J* = 8.56, 2H, H2' and H6'); 7.64 (d, *J* = 8.52, 2H, H2" and H6"); 7.17 (d, *J* = 2.76, 1H, H H3); 6.98 (d, *J* = 2.76, 1H, H4). 6.61 (d, *J* = 8.56, 2H, H3' and H5'); 5.82 (s, 2H, NH_2); ^{13}C NMR: 162.85 (CO); 153.02 (C-5); 152.08 (C-4'); 149.48 (C-2); 136.10 (CH); 131.74 (C-3" and C-5"); 129.14 (C-1"); 128.47 (C-2' and C-6'); 125.54 (C-2" and C-6"); 120.90 (C-4"); 118.94 (C-1'); 115.04 (C-4); 112.40 (C-3'and C-5'); 108.80 (C-3).

2-Hydroxy-benzoic acid 5-[4-bromophenyl]-furan-2-ylmethylene-hydrazide (6c): Yellow powder; Yield 90.12 %; m.p. 261-263 °C; IR (cm^{-1}): 3450 ν(O–H); 3248 ν(N–H); 1621 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.85 (s, 2H, OH and NH); 8.38 (1H, s, CH=N); 7.87 (d.d, *J* = 6.6, 1H, H6'); 7.76 (d, *J* = 8.49, H3" and H5"); 7.68 (d, *J* = 8.79, H2" and H6"); 7.47-7.41 (t.d, *J* = 7.56, *J* = 1.38, 1H, H4'); 7.23 (d, *J* = 3.57, H3); 7.10 (d, *J* = 3.75, H4); 6.99-6.93 (m, 2H, H3' and H5'). ^{13}C NMR: 164.27 (CO); 158.53 (C-2'); 153.45 (C-5); 148.81 (C-2); 133.43 (C-4'); 131.63 (C-3" and C-5"); 128.23 (C-1"); 128.12 (C-6'); 125.53 (C-2" and C-6"); 120.98 (C-4"); 118.56 (C-5'); 116.87 (C-3'); 116.34 (C-4); 115.60 (C-1'); 108.79 (C-3).

2-Amino-benzoic acid 5-[4-bromophenyl]-furan-2-ylmethylene-hydrazide (6d): White fine plates; Yield 90.18 %; m.p.213-214 °C; IR (cm^{-1}): 3216 ν(N–H); 1627 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.67 (1H, s, NH); 8.32 (1H, s, CH=N); 7.74 (d, *J* = 8.8, H3" and H5"); 7.66 (d, *J* = 8.04, H2" and H6"); 7.58 (d, *J* = 8.08, 1H, H6'); 7.21 (t, *J* = 8.04, 1H, H4'); 7.18 (d, *J* = 3.68, H3); 7.02 (d, *J* = 3.68, H4); 6.78 (d, *J* = 8.04, 1H, H3'); 6.58 (t, *J* = 7.36, 1H, H5'); 6.42 (s, 2H, NH_2); ^{13}C NMR:165.90 (CO); 153.99 (C-5); 150.20 (C-2'); 150.65 (C-2); 136.84 (CH); 132.91 (C-4'); 132.53 (C-3" and C-5"); 129.28 (C-1"); 128.86 (C-6'); 126.37 (C-2" and C-6"); 121.77 (C-4"); 116.97 (C-5'); 116.18 (C-3'); 115.20 (C-4); 113.91 (C-1'); 109.64 (C-3).

4-Methyl-benzenesulfonyl (5-[4-bromophenyl]-furan-2-ylmethylene)-hydrazide (6e): Brown powder; Yield 85.20 %; m.p.168-170 °C; IR (cm^{-1}): 3179 ν(N–H); 1323-1171 ν(S (=O)$_2$); ^{1}H NMR (DMSO-d_6): 11.53 (1H, s, NH); 7.81 (1H, s, CH=N); 7.78 (d, *J* = 8.08, 2H, H2' and H6'); 7.67 (d, *J* = 8.8, H3" and H5"); 7.64 (d, *J* = 8.8, H2" and H6"); 7.41 (d, *J* = 8.04, 2H, H3' and H5'); 7.11 (d, *J* = 3.68, H3); 6.92 (d, *J* = 3.64, H4); 2.33 (s, 3H, CH_3); ^{13}C NMR: 153.99 (C-5); 149.16 (C-2); 144.08 (C-4'); 137.16 (CH); 136.68 (C-1'); 132.54 (C-3" and C-5"); 130.26 (C-3' and C-5'); 129.11 (C-1"); 127.73 (C-2" and C-6"); 126.38 (C-2' and C-6'); 121.87 (C-4"); 116.51 (C-4); 109.45 (C-3), 21.55 (CH_3).

4-Hydroxy-benzoic acid thiophen-2-ylmethylene-hydrazide (7a): Beige powder; Yield 66.91 %; m.p.254-255 °C; IR (cm^{-1}): 3390 ν(O–H); 3252 ν(N–H); 1634 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.62 (1H, s, NH); 10.14 (s, 1H, OH); 8.65 (1H, s, CH=N); 7.80 (d, *J* = 8.08, 2H, H2' and H6'); 7.64 (d, *J* = 5.12, 1H, H5); 7.34 (d, *J* = 2.92, 1H, H3); 7.12 (t, *J* = 4.4, 1H, H4); 6.87 (d. *J* = 8.04, 2H, H3' and H5'); ^{13}C NMR: 163.23 (CO); 161.24 (C-4'); 142.60 (CH); 139.93 (C-2' and C-6'); 131.06, 130.19 (C-3 and C-4); 129.18 (C-1'); 128.37 (C-3' and C-5'); 124.38, 115.59 (C-2 and C5).

4-Amino-benzoic acid thiophen-2-ylmethylene-hydrazide (7b): Beige powder; Yield 55.39 %; m.p.176-177 °C; IR (cm^{-1}): 3205 ν(N–H); 1620 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.43 (1H, s, NH); 8.62 (1H, CH=N); 7.66 (d, *J* = 8.8, 2H, H2' and H6'); 7.62 (d, *J* = 5.16, 1H, H5); 7.40 (d, *J* = 5.16, 1H, H3); 7.12 (t, *J* = 3.64, 1H, H4); 6.60 (d, *J* = 8.08, 2H, H3' and H5'); 5.79 (s, 2H, NH_2); ^{13}C NMR: 163.45 (CO); 152.85 (C-4'); 141.68 (CH); 140.19, 130.63 (C-3 and C-4); 129.89, 128.86 (C-2 and C-5); 128.32 (C-2' and C-3'); 119.99 (C-1'); 113.15 (C-3' and C-5').

2-Hydroxy-benzoic acid thiophen-2-ylmethylene-hydrazide (7c): Beige powder; Yield 92.74 %; m.p.241-243 °C; IR (cm^{-1}): 3469 ν(O–H); 3245 ν(N–H); 1622 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.82 (s, 2H, NH, OH); 7.87 (d, *J* = 8.08, 1H, H6'); 7.71 (d, *J* = 5.12, 1H, H5); 7.50 (d, *J* = 3.68, 1H,

H3); 7.44 (t, *J* = 8.08, 1H, H4'); 7.16 (t, *J* = 4.4, 1H, H4); 6.98 (d, *J* = 8.08, 1H, H3'); 6.95 (t, *J* = 8.08, 1H, H5'); ^{13}C NMR:165.12 (CO); 159.49 (C-2'); 144.30, 131.98 (C-3 and C-4); 139.39 (CH); 134.35 (C-4'); 129.88, 129.05 (C-2 and C5); 128.50 (C-6'); 119.50 (C-5'); 117.82 (C-3'); 116.51 (C-1').

2-Amino-benzoic acid thiophen-2-ylmethylene-hydrazide (7d): Beige powder; Yield 51.02 %; m.p.174-175 °C; IR (cm^{-1}): 3176 ν(N–H); 1635 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 11.59 (1H, s, NH); 8.59 (1H, s, CH=N); 7.65 (d, *J* = 5.16, 1H, H5); 7.55 (d, *J* = 8.04, 1H, H6'); 7.43 (d, *J* = 3.68, 1H, H3); 7.20 (t, *J* = 8.08, 1H, H4'); 6.77 (d, *J* = 8.08, 1H, H3'); 7.14-7.12 (d.d, *J* = 5.14, *J* = 3.68, 1H, H4); 6.57 (t, *J* = 8.08, 1H, H5'); 6.37 (s, 2H, NH_2); ^{13}C NMR:165.72 (CO); 150.56 (C-2'); 142.48, 139.94 (C-3 and C-4); 132.82 (C-4'); 131.05 (CH), 129.20, 128.83 (C-2 and C-5); 128.38 (C-6'); 116.93 (C-5'); 115.18 (C-3'); 114.01 (C-1').

4-Methyl-benzenesulfonyl(thiophen-2-ylmethylene)-hydrazide (7e): Beige powder; Yield 78.75 %; m.p.144-146 °C; IR (cm^{-1}): 3163 ν(N–H); 1341-1168 ν(S (=O)$_2$); ^{1}H NMR (DMSO-*d*$_6$): 11.39 (1H, s, NH); 8.10 (1H, s, CH=N); 7.85 (d, *J* = 5.12, 1H, H5); 7.75 (d, *J* = 8.8, 2H, H2' and H6'); 7.40 (d, *J* = 8.04, 2H, H3' and H5'); 7.35 (d, *J* = 3.68, 1H, H3); 7.06 (d.d, *J* = 5.12, *J* = 3.68, 1H, H4) 2.34 (s, 3H, CH_3); ^{13}C NMR: 142.78 (C-4'); 138.83 (CH); 136.57 (C-1'); 144.05, 131.30 (C-3 and C-4); 130.19 (C-3' and C-5'); 129.26, 128.34 (C-2 and C-5); 127.76 (C-2' and C-6'); 21.55 (CH_3).

4-Amino-benzoic acid (1*H*-pyrrol-2-ylmethylene)-hydrazide (8b): Brown powder; Yield 94.26 %; m.p.175-176 °C; IR (cm^{-1}): 3225 ν(N–H); 1612 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 11.46 (1H, s, NH); 11.25 ($NH_{pyrrole}$); 7.69 (d, *J* = 8.56, 2H, H2' and H6'); 6.90 (s, 1H, H5); 6.44 (s, 1H, H3); 6.13 (s, 1H, H4); 6.63 (d, *J* = 8.56, 2H, H3' and H5'); 5.75 (s, 2H, NH_2).

2-Hydroxy-benzoic acid (1*H*-pyrrol-2-ylmethylene)-hydrazide (8c): m.p. 222-224C; IR (cm^{-1}): 3384 ν(O–H); 3268 ν(N–H); 1618 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$):12.11 (s, 1H, OH); 11.59 (2H, s, NH and $NH_{pyrrole}$); 6.96 (m, 1H, H5); 7.89 (d, *J* = 8.07, 1H, H6'); 7.44 (t, *J* = 7.71, 1H, H4'); 6.96-6.91 (m, 2H, H3' and H5'); 6.52 (s, 1H, H3); 6.15 (s, 1H, H4).

2-Amino-benzoic acid (1*H*-pyrrol-2-ylmethylene)-hydrazide (8d): m.p. 145-147 °C; ^{1}H NMR (DMSO-*d*$_6$): 11.47 (1H, s, NH); 11.30 (1H, s, $NH_{pyrrole}$): 8.80 (1H, s, CH=N); 7.52 (d, 1H, H6'); 7.17 (t, *J* = 7.71, 1H, H4'); 6.88-6.13 (m, 5H, H3', H5', H3, H4 andH5); 5.83 (s, 2H, NH_2).

4-Methyl-benzenesulfonyl(1*H*-pyrrol-2-ylmethylene)-hydrazide (8e): Brown powder, Yield 84.56 %; m.p. 188-189 °C; IR (cm^{-1}): 3171 ν(N–H); 1332-1180 ν(S (=O)$_2$). ^{1}H NMR (DMSO-*d*$_6$): 11.27 (1H, s, NH); 10.93 ($NH_{pyrrole}$); 6.85 (s, 1H, H5); 6.35 (d, *J* = 1.11, 1H, H3); 6.08 (t, *J* = 1.47, 1H, H4); 7.80 (d, *J* = 8.08, 2H, H2' and H6'); 7.38 (d, *J* = 8.04, 2H, H3' and H5'); 2.35 (s, 3H, CH_3).

Furan-2-carboxylic acid (4-hydroxy-benzylidene)-hydrazide (9a): Brown powder, Yield 68.24 %; m.p. 113-121 °C; IR (cm^{-1}): 3394 ν(O–H); 3161 ν(N–H); 1596 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 11.67 (1H, s, NH); 9.95 (s, 1H, OH); 8.35 (1H, s, CH=N); 7.91 (s, 1H, H5); 7.56 (d, *J* = 8.8, 2H, H2' and H6'); 7.27 (s, 1H, H3); 6.85 (d, *J* = 8.08, 2H, H3' and H5').6.68 (s, 1H, H4); ^{13}C NMR:160.02 (C-4'); 154.62 (CO); 148.80 (CH); 147.36 (C-2); 146.15 (C-5); 129.44 (C-2' and C-6'); 125.78 (C-1'); 116.29 (C-3' and C-5'); 115.15 (C-3); 112.58 (C-4).

Furan-2-carboxylic acid (4-nitro-benzylidene)hydrazide (9b): Yellow powder; Yield 96.24 %; m.p.184-176 °C; IR (cm^{-1}): 3278 ν(N–H); 1675 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 12.15 (1H, s, NH); 8.54 (1H, s, CH=N); 8.31 (d, *J* = 8.8, 2H, H3' and H5'); 7.98 (s, 1H, H5); 7.96 (d, *J* = 8.8, 2H, H2' and H6'); 7.36 (s, 1H, H3); 6.73 (d.d, *J* = 3.68, *J* = 1.48, 1H, H4); ^{13}C NMR:154.89 (CO); 150.10 (C-4'); 148.38 (CH); 146.79 (C-2); 145.85 (C-5); 141.09 (C-1'); 128.56 (C-2' and C-6'); 124.65 (C-3' and C-5'); 116.15 (C-3); 112.78 (C-4).

Furan-2-carboxylic acid (2,4-dihydroxy-benzylidene)-hydrazide (9c): Brown powder; Yield 85.29 %; m.p.173-175 °C; IR (cm^{-1}): 3453 ν(O–H); 3117 ν(N–H); 1630 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 11.95 (1H, s, NH); 11.36 (s, 1H, OH); 9.98 (s, 1H, OH); 8.52 (1H, s, CH=N); 7.93 (s, 1H, H5); 7.27 (d, 1H, H3); 7.31 (d, *J* = 9.52, 1H, H6'); 6.37 (d, *J* = 8.08, 1H, H5'); 6.69 (t, *J* = 1.44, 1H, H4); 6.33 (s, 1H, H3'); ^{13}C NMR: 161.31 (C-4'); 159.98 (C-2'); 154.31 (CO); 149.76 (CH); 146.99 (C-2); 146.35 (C-5); 131.80 (C-6'); 115.46 (C-3); 112.67 (C-4); 111.12 (C-1'); 108.31 (C-5'); 103.22 (C-3').

Furan-2-carboxylic acid (2,4-dinitro-benzylidene)-hydrazide (9d): Yellow powder; Yield 39.54 %; m.p.147-149 °C; IR (cm^{-1}): 3100 ν(N–H); 1660 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 12.47 (1H, s, NH); 8.90 (1H, s, CH=N); 8.60 (d.d, *J* = 8.08, *J* = 2.2, 1H, H5'); 8.38 (d, *J* = 8.8, 1H, H6'); 8.01 (s, 1H, H5); 7.41 (s, 1H, H3); 7.80 (d, *J* = 2.2, 1H, H3'); 6.75-6.73 (d.d, *J* = 3.28, *J* = 2.2, H4).

Furan-2-carboxylic acid furan-2-ylmethylene-hydrazide (9e): Beige powder; Yield 77.73 %; m.p.110-111 °C; IR (cm^{-1}): 3106 ν(N–H); 1642 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 11.83 (1H, s, NH); 8.39 (1H, s, CH=N); 7.94 (s, 1H, H5); 7.85 (s, 1H, H5'); 7.27 (s, 1H, H3); 6.93 (d, *J* = 3.64, 1H, H3'); 6.70 (d, *J* = 1.44, 1H, H4); 6.63 (t, *J* = 1.48, 1H, H4'); ^{13}C NMR: 154.67 (CO); 149.94 (C-2'); 147.15 (C-2); 146.37 (C-5); 145.76 (C-5'); 138.15 (CH); 115.58 (C-3); 114.12 (C-3'); 112.78 (C-4'); 112.68 (C-4).

Furan-2-carboxylic acid (5-nitro-furan-2-ylmethylene)-hydrazide (9f): Yellow powder; Yield 68.22 %; m.p. 149-151 °C; IR (cm^{-1}): 3129 ν(N–H); 1668 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 12.27 (1H, s, NH); 8.39 (CH=N); 7.98 (s, 1H, H5); 7.79 (d, *J* = 3.68, 1H, H4'); 7.35 (s, 1H, H3); 7.26 (d, *J* = 4.4, 1H, H3'); 6.73-6.72 (d.d, *J* = 3.68, *J* = 1.48, 1H, H4); ^{13}C NMR: 154.82 (CO); 152.44 (C-5'); 152.24 (C-2'); 146.92 (C-2); 146.63 (C-5); 136.03 (CH); 116.51 (C-3); 115.19 (C-3'); 115.80 (C-4'); 112.87 (C-4).

Thiophene-2-carboxylic acid (4-hydroxy-benzylidene)-hydrazide (10a): Beige powder; Yield 65.52 %; m.p. 278-282 °C; IR (cm^{-1}): 3654 ν(O–H); 3267 ν(N–H); 1625 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$):11.69 (1H, s, NH); 9.97 (1H, s, OH); 7.64 (d, *J* = 8.8, 2H, H2' and H6'); 8.06 (m, 2H, H3 and H5); 7.21 (t, *J* = 3.64, 1H, H4); 6.86 (d, *J* = 8.08, 2H, H3' and H5').

Thiophene-2-carboxylic acid (4-nitro-benzylidene)-hydrazide (10b): Yellow powder; Yield 92.60 %; m.p. 247-248 °C; IR (cm^{-1}): 3163 ν(N–H); 1648 ν(C=O); ^{1}H NMR (DMSO-*d*$_6$): 12.15 (1H, s, NH); 8.30 (d, *J* = 8.76, 2H, H3' and H5'); 8.15 (1H, s, CH=N); 8.06 (m, 4H, H2', H6', H5 and H3); 7.24 (t, *J* = 3.64, 1H, H4); ^{13}C NMR: 158 (CO); 145.49 (C-4');

142.37 (C-5); 138.33 (CH); 135.73 (C-4); 133.02 (C-2); 130.06 (C-1'); 128.61 (C-2' and C-6'); 127.40 (C-3); 124.66 (C-3' and C-5').

Thiophene-2-carboxylic acid (2,4-dihydroxy-benzylidene)hydrazide (10c): Beige powder; Yield 82.26 %; m.p. 252-253 °C; IR (cm^{-1}): 3682 ν(O–H); 3120 ν(N–H); 1636 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.94 (1H, s, NH); 11.30 (s, 1H, OH); 9.97 (s, 1H; OH); 7.89 (d, *J* = 3.68, 1H, H5); 7.86 (d, *J* = 5.12, 1H, H3); 7.33 (d, *J* = 8.76, 1H, H6'); 6.36 (s, 1H, H3'); 6.34 (d, *J* = 8.8, 1H, H5').7.22 (t, *J* = 4.4, 1H, H4); ^{13}C NMR: 161.32 (C-4'); 159.92 (C-2'); 157.81 (CO); 149.23 (C-2); 138.43 (CH); 132.35 (C-3); 131.66 (C-5); 129.42 (C-6'); 128.70 (C-4); 111.15 (C-1'); 108.30 (C-5'); 103.20 (C-3').

Thiophene-2-carboxylic acid (2,4-dinitro-benzylidene)hydrazide (10d): Yellow powder; Yield 96.26 %; m.p. 244-246 °C; IR (cm^{-1}): 3171 ν(N–H); 1645 ν(C=O). ^{1}H NMR (DMSO-d_6): 12.43 (1H, s, NH); 8.80 (s, 1H, H3'); 8.79 (1H, s, CH=N); 8.62 (d, *J* = 8.8, 1H, H5'); 8.41 (d, *J* = 8.8. 1H, H6'); 8.01 (s, 1H, H5); 7.97 (d, *J* = 4.4, 1H, H3); 7.25 (t, *J* = 4.4, 1H, H4).

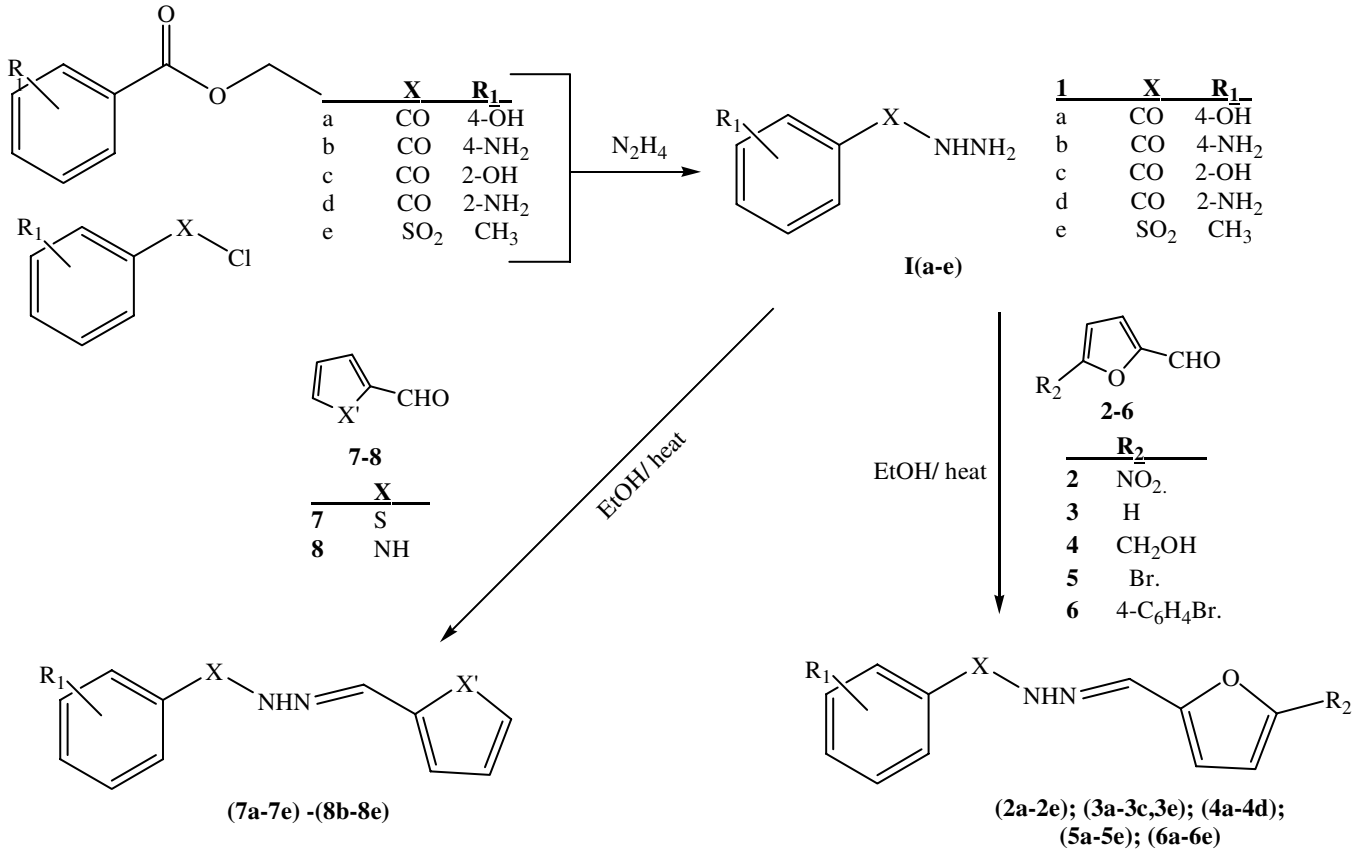
Thiophene-2-carboxylic acid furan-2-ylmethylene-hydrazide (10e): Beige powder, Yield 70.02 %; m.p. 163-165 °C; IR (cm^{-1}): 3160 ν(N–H); 1658 ν(C=O); ^{1}H NMR (DMSO-d_6): 11.82 (1H, s, NH); 8.32 (1H, s, CH=N); 8.08 (s, 1H, H5); 7.99 (s, 1H, H3); 7.87 (s, 1H, H5'); 7.21 (s, 1H, H4); 6.95 (d, *J* = 2.92, 1H, H3'); 6.64 (s, 1H, H4'); ^{13}C NMR: 158.24 (CO); 149.88 (C-2); 145.80 (C-2'); 145.43 (C-5'); 138.71 (CH); 137.88 (C-3); 134.21 (C-5); 129.50 (C-4); 114.21 (C-3'); 112.80 (C-4').

Thiophene-2-carboxylic acid (5-nitro-furan-2-yl-methylene)-hydrazide (10f); Yellow powder; Yield 96.79 %; m.p.259-260 °C; IR (cm^{-1}): 3146 ν(N–H); 1651 ν(C=O); ^{1}H NMR (DMSO-d_6): 12.24 (1H, s, NH); 8.36 (1H, s, CH=N); 7.78 (d, *J* = 3.68, 1H, H4'); 7.26 (d, *J* = 4.4, 1H, H3'); 7.24-7.22 (m, 3H, H3, H4 and H5); ^{13}C NMR: 158.50 (CO); 152.40 (C-5'); 152.24 (C-2'); 138.05 (C-2); 135.71 (C-3); 132.00 (C-5); 130.29 (C-4); 127.63 (C-4); 115.89 (C-4'); 115.22 (C-3').

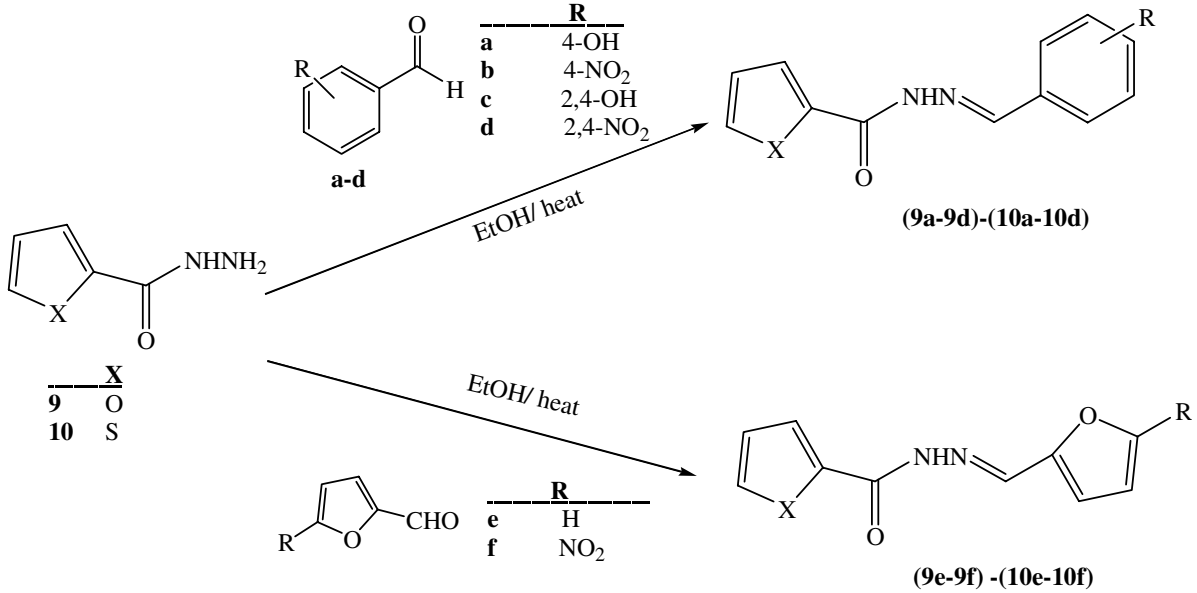
Inhibition zone (IZ): Preliminary experiments were carried out to determine antimicrobial activities of all compounds *in vitro* against two Gram-positive bacteria [*Staphylococcus aureus* (ATCC 25923); *Bacillus subtilis* (ATCC 6633)]; three Gram-negative bacteria [*EC*: *Escherichia coli* (ATCC 25922); *ST*: *Salmonella susi* (ATTC 13070); *PA*: *Pseudomonas aeruginosa* (ATCC27853)] and one Fungus [*Candida albicans* (ATCC 10231)], using wells diffusion method according to National Committee for Clinical Laboratory Standards. Petri plates containing 20 mL of nutrient agar. Wells (6 mm in diameter) were divided into agar and all compound were tested in a concentration of 100 µg/mL and incubated at 37°C (bacterial strains) and at 25 °C (fungal strains) for 24 h. The antimicrobial activities were determined based on measurement of the diameter of inhibition zone formed around the well using gentamicin as reference drug. The compounds were dissolved in DMSO that did not interfere in the microorganism's growth.

RESULTS AND DISCUSSION

Synthesis of nifuroxazide analogues: Total of 44 hydrazide-hydrazone derivatives have been synthesized, some among them were novel and some were new. Nifuroxazide itself (**2a**)



Scheme-I: Synthetic pathway for compounds (**1a-1d**)-(**8b-8e**)



Scheme-II: Synthetic pathway for compounds (**9a-9f**)-(**10a-10f**)

TABLE-1
INHIBITION ZONE VALUES (mm) OF COMPOUNDS **1(a-e)-8(a-e)**, **9(a-f)** AND **10(a-f)**

Compd. No.	Gram-positive		Gram-negative			Fungous	Compd. No.	Gram-positive		Gram-negative			Fungous
	SA	BS	EC	SS	PA	CA		SA	BS	EC	SS	PA	CA
1a	0	0	0	12	13	0	**6c****	0	0	0	15	10	0
1b	0	0	0	12	15	0	**6d****	0	0	14	14	15	0
1c	0	0	0	0	0	0	**6e**	0	0	0	15	10	0
1d	0	15	10	10	10	0	**7a**	0	0	0	12	15	0
1e	0	0	0	20	0	0	**7b**	10	0	0	14	15	0
2a	16	20	15	12	16	13	**7c**	0	0	0	0	15	0
2b	25	27	22	16	0	30	**7d**	0	0	12	0	14	0
2c	15	20	0	15	15	15	**7e**	0	10	20	10	20	0
2d	30	20	20	18	0	30	**8a**	NT	NT	NT	NT	NT	NT
2e	20	20	15	10	18	15	**8b****	0	0	0	16	0	0
3a	0	0	0	15	15	0	**8c**	0	0	0	15	15	0
3b	10	0	12	0	15	0	**8d**	NT	NT	NT	NT	NT	NT
3c	0	0	0	0	15	0	**8e**	0	0	0	15	0	0
3d	NT	NT	NT	NT	NT	NT	**9a****	0	0	0	15	0	0
3e	0	0	0	15	12	0	**9b**	0	0	0	12	0	0
4a**	0	0	0	12	12	0	**9c**	0	0	0	20	0	0
4b**	0	0	0	9	12	0	**9d****	0	13	13	10	0	0
4c	0	0	0	15	17	0	**9e**	0	0	0	13	0	0
4d*	0	0	0	15	0	0	**9f**	20	26	19	20	13	0
4e	NT	NT	NT	NT	NT	NT	**10a**	0	0	0	12	0	0
5a**	0	0	10	12	10	0	**10b****	0	12	0	14	0	0
5b*	10	0	0	12	10	0	**10c**	0	0	11	12	10	0
5c	0	0	0	12	16	0	**10d****	0	0	10	15	15	0
5d**	0	0	12	14	15	0	**10e**	0	0	0	15	0	0
5e*	0	0	0	20	10	0	**10f**	25	22	15	15	11	0
6a	0	12	10	17	10	0	Gen	27	34	23	NT	36	NT
6b*	16	15	10	15	0	0	–	–	–	–	–	–	–

NT = Not tested; Gentamicin. SA = *Staphylococcus aureus* (ATCC 25923); BS = *Bacillus subtilis* (ATCC 6633); EC = *Escherichia coli* (ATCC 25922); ST = *Salmonella susi* (ATTC 13070); PA = *Pseudomonas aeruginosa* (ATCC27853); CA = *Candida albicans* (ATCC 10231); *Novel compounds; ** New compounds.

and other representative analogues of the same structural type [(**2a-2e**)-(**6a-6e**)] were synthesized by condensation of 5-substituted furfural with substituted benzene hydrazide and benzenesulfonyl hydrazide. Other nifuroxazide analogues series [(**7a-7e**)-(**8b-8e**)] were synthesized by replacing the furan ring with thiophene and pyrrole. Unfortunately, compounds (**3d**, **4e** and **8a**) have not been synthesized due to inadequacy of starting materials. Furthermore, other modeling nifuroxazide structures [**9(a-f)**-(**10a-10f**)] were also prepared from the reaction of furoic hydrazide and the corresponding hydrazide of thiophene with a variety of aromatic aldehydes, furan-2-carboxaldehyde and thiophene-2-carboxaldehyde. The

compounds were structurally identified through IR, ^{1}H NMR and ^{13}C NMR spectra. The synthesis of target compounds is outlined in **Schemes I** and **II**.

Antimicrobial activity determination: Inhibition zone (IZ), was determined for all compounds, hydrazide analogues and hydrazide-hydrazones analogues, by well diffusion method, using gentamycin as reference drug against two Gram-positive bacteria [*Staphylococcus aureus* (ATCC 25923); *Bacillus subtilis* (ATCC 6633)]; three Gram-negative bacteria [EC: *Escherichia coli* (ATCC 25922); ST: *Salmonella susi* (ATTC 13070); PA: *Pseudomonas aeruginosa* (ATCC27853)] and one Fungus [*Candida albicans* (ATCC 10231)].

Based on inhibition zone (Table-1), some interesting observation can be made. Nitrofuran analogs (**2a-2d**) exhibited remarkable activity against Gram-positive bacteria in the comparison with Gram-negative bacteria and showed good activity against fungus. The most active compound in this series was 4-amino-benzoic acid (5-nitro-furan-2-ylmethylene)-hydrazide (**2b**) and 2-amino-benzoic acid (5-nitro-furan-2-ylmethylene)-hydrazide (**2d**).

The inhibition zone for substituted benzoic acid (furan/thiophene/pyrrole-2-ylmethylene)hydrazide analogs showed less activity against tested microorganism. Furthermore, the inhibition zone for compounds (**9a-9g**) and (**10a-10g**) showed no activity against tested microorganism with two exceptions (**9f**) and (**10f**) suggesting that using furoic hydrazide and the corresponding hydrazide of thiophene did not improve the antimicrobial profile for this type of compounds. Regarding the activity against *Candida albicans,* all compounds showed no activity with an exception of nitro furan substituted (**2a-2d**).

Conclusion

A series of hydrazide-hydrazone analogues, which are structurally-related to known "nifuroxzide" antimicrobial drugs, were synthesized, structurally identified and tested for antimicrobial activity against a panel of Gram-positive and Gram-negative bacteria and the yeast-like pathogenic fungus *Candida albicans*. The result of the antimicrobial testing revealed that most compounds showed activity against one or more of selected Gram-negative bacteria. Compound **2b** showed almost the same activity that showed by gentamycin. Regarding the activity against *Candida albicans*, all compounds showed no activity with an exception of substituted nitro furan (**2a-2e**).

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