WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 01, 1215-1225. Research Article ISSN 2277– 7105

SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF (*E*)-N'-(SUBSTITUTED-BENZYLIDENE) -2-(2-CHLORO-4-FLUOROPHENYL) ACETOHYDRAZIDE DERIVATIVES

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Article Received on
09 Nov 2015,

Revised on 29 Nov 2015,
Accepted on 22 Dec 2015

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ABSTRACT

The present paper describes the synthesis of (*E*)-N'-(3,4,5-trimethoxybenzylidene)-2-(2-chloro-4-fluorophenyl)acetohydrazide derivatives **4a-k**, prepared in a three-steps from commercially available 2-(2-chloro-4-fluorophenyl)acetic acid **1.** The structural determination of the newly synthesized acetohydrazide-hydrazones was confirmed by analytical tools like ^{1}H NMR, mass and IR. These compounds were evaluated against a panel bacterial and fungal pathogens such as Gram-positive (*Staphylococcus.pyogens* and *Staphylococcus aureus)*,Gram-negative (*Escherichia coli* and *Pseudomonas .aeruginosa*) and *Aspergillus niger* and *Candida albicans* with reference to Chloramphenicol and Nystatin as the standard drug. Compounds **4a-k** showed moderate to good antibacterial activity but displayed weak fungal activity.

KEYWORDS: Acetohydrazides, Antibacterial activity, Hydrazones, Chloramphenicol, Rotamers.

1. INTRODUCTION

Hydrazide-hydrazones are well thought-out to be good candidates for diverse pharmaceutical applications and the therapeutic prominence has been well recognized. In addition,

hydrazide-hydrazones were reported to bring out anticancer[1–8] and antiHIV properties[9] and consequently they have occupied an important place in medicinal chemistry.

Resistance to a number of antimicrobial agents by a range of pathogenic bacteria is becoming a most important worldwide problem. The prevalent use and exploitation of antibiotics is one of the cause accredited to the materialization of drug resistance to preponderance of antibacterial agents[10]. It necessitates the scientific society to expand new antimicrobial agents with potent and broad spectrum of antimicrobial action against resistant pathogens. Consequently there is a ample scope on research on newer antibacterial agents.

Examples of some of the hydrazide-hydrazone derivatives exhibiting antibacterial activity is presented in **Figure-1**. The antibacterial activity of hydrazide-hydrazone derivatives **I** against various pathogenic bacterial strains[11], Benzimidazole derivatives with hydrazone moiety **II**[12], chloropyrrole derivatives of aroylhydrazone **III**[13] and.Vanillin based hydrazines **IV**, **V** showed antibacterial activity against S. aureus and P. aeruginosa.[14] Furthermore, hydrazones **VI** were act as selective inhibitors of S. aureus β-ketoacyl carrier proteinsynthase-3.[15]

With the aim of obtaining novel hydrazide-hydrazones with an extensive range of pharmaceutical applications, we report herein the synthesis and antimicrobial activity of a series of hydrazide-hydrazones.

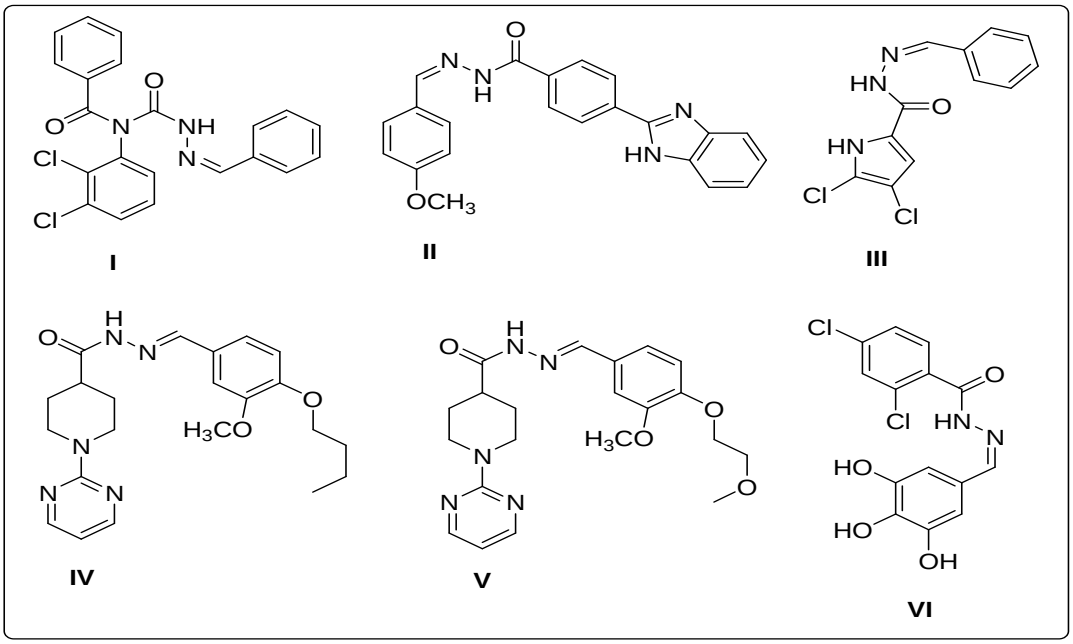


Figure 1: Examples of some hydrazone derivatives exhibiting antimicrobial activity

2.0 MATERIALS AND METHODS

The solvents were purified according to standard procedures prior to use, and all commercial chemicals were used as received. For thin-layer chromatography (TLC) analysis, Merck pre-coated Plates (silica gel 60 F254) were used and spots were visualized with UV light. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography and the eluting solvents are indicated in the procedures. Melting point (mp) determinations were performed by using Mel-temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded in Varian MR-400 MHz instrument. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal standard and the signals were reported as s (singlet), d (doublet), dd (doblet of doblet), t (triplet), q (quartet), m (multiplet) and coupling constants in Hz. The mass spectra were recorded on Agilent ion trap MS. Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer.

2.1 EXPERIMENTAL

2.1.1 Preparation of ethyl 2-(2-chloro-4-fluorophenyl)acetate (2)

To a solution of compound **1 (**2 g, 10.60 mmol) in ethanol (10 mL) was added sulphuric acid (3-4 drops) and refluxed for 5 h. After completion of the reaction, ethanol was evaporated under reduced pressure and the obtained residue was taken in ethylacetate (25 mL) and washed with 10% aq; $NaHCO_3$ solution (2 x15 ml) followed by water wash and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to afford ethyl 2-(2-chloro-4-fluorophenyl)acetate **2.** Yellow oily liquid, Yield: 1.72 g, 75%; B.p: 165-167°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36 (d, J = 7.2 Hz, 1H), 7.18 (d, J = 7.0 Hz, 1H), 7.0 (d, J = 7.0 Hz, 1H), 6.70 (brs, 1H), 3.90 (brs, 2H), 3.62 (s, 2H); ESI MS: m/z, 202.9 (M+1).

2.1.2 Preparation of 2-(2-chloro-4-fluorophenyl)acetohydrazide (3)

To a solution of ethyl 2-(2-chloro-4-fluorophenyl)acetate **2** (1 g, 4.61 mmol) in ethanol (15.0 mL) was added hydrazine hydrate (1.38g, 27.66 mmol) and heated to reflux for 8 h. After completion of the reaction, ethanol was concentrated under reduced pressure to obtain crude compound **3**. The crude compound was slurred in n-Hexane, filtered at the vaccum pump and dried to obtain 2-(2-chloro-4-fluorophenyl)acetohydrazide **3**. Pale yellow solid, Yield: 0.75g, 80%; M.p 118-119 ^{o}C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36 (d, J = 7.2 Hz, 1H), 7.18 (d, J = 7.0 Hz, 1H), 6.94 (d, J = 7.0 Hz, 1H), 4.20 (q, J = 5.6 Hz, 2H), 3.78 (s, 2H), 1.26 (t, J = 5.6 Hz, 3H).

ESI-MS: m/z, 293.8 (M+1).

2.1.3 General Experimental Procedure for the Synthesis of Hydrazone derivatives 4a-k

To a stirred solution of 2-(2-chloro-4-fluorophenyl)acetohydrazide **3** (100 mg, 0.493 mmol) in ethanol was added corresponding benzaldehydes **a-k** (0.518 mmol) and refluxed for 1 h. After completion of the reaction, the reaction mixture was cooled to room temperature and the precipitated solids were filter and washed with pet-ether and dried at the pump to obtain corresponding aceto-hydrazide-hydrazone products **4a-k** . The yields of the products varied from 82 – 90%. The spectral characteristic data of the individual products is given below.

2.1.3.1 (*E*)-N'-(4-hydroxybenzylidene)-2-(2-chloro-4-fluorophenyl)acetohydrazide (4a)

White solid; Yield: 84%; M.p: 109-110 ^{o}C; IR (KBr): υ_{max} 3240, 3068, 3038, 2926, 1649, 1595, 1539, 1514, 1491, 1431, 1402, 1372, 1347, 1309, 1279, 1246, 1210, 1167, 1120, 1110, 1068, 1044, 990, 967, 932, 907, 897, 873, 859, 837, 807, 795, 752, 690, 667, 607, 581, 570, 529, 498 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 11.40 (* 11.28, s, 1H), 10.84 (s, 1H), 8.10 (* 7.90, s, 1H), 7.58 (d, J = 7.2 Hz, 2H), 7.50-7.40 (m, 2H), 7.22-7.16 (m, 1H), 6.80 (d, J = 7.2 Hz, 1H), 4.20 (*3.70, s, 2H); ESI MS: m/z, 306.9 (M+1)$^{+}$;

2.1.3.2 (*E*)-N'-(4-nitrobenzylidene)-2-(2-chloro-4-fluorophenyl)acetohydrazide (4b)

Yellow solid; Yield: 86%; M.p: 88-89 ^{o}C;

^{1}H NMR (500 MHz, DMSO-d$_6$): δ 11.98 (* 11.78, s, 1H), 8.36 (* 8.18, s, 1H), 8.24 (d, J = 6.8 Hz, 2H), 7.98 (d, J = 6.8 Hz, 2H), 7.52-7.44 (m, 2H), 7.20 9d, J = 7.2 Hz, 1H), 4.20 (*3.78, s, 2H); ESI MS: m/z, 334 (M-1)$^{+}$;

2.1.3.3(*E*)-N'-(4-(methylsulfonyl)benzylidene)-2-(2-chloro-4-luorophenyl)acetohydrazide (4c)

Off-white solid; Yield: 86%; M.p:121-122 ^{o}C; IR (KBr): υ_{max} 3434, 3247, 3220, 3065, 3013, 2962, 2930, 2859, 1672, 1606, 1594, 1548, 1494, 1415, 1401, 1352, 1340, 1310, 1296, 1257, 1235, 1206, 1177, 1141, 1084, 1046, 1015, 982, 959, 926, 894, 881, 863, 840, 827, 801, 786, 772, 722, 691, 679, 639, 620, 577, 550, 536, 528, 493 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 11.82 (* 11.76, s, 1H), 8.36 (* 8.18, s, 1H), 8.0 (brs, 4H),7.52 -7.44 (m, 2H), 7.20 (d, J = 7.2 Hz, 1H), 4.20 (* 3.78, s, 2H), 3.22 (s, 3H); ESI MS: m/z, 368.9 (M+1)$^{+}$;

2.1.3.4(*E*)-N'-(4-(trifluoromethoxy)benzylidene)-2-(2-chloro-4-fluorophenyl) acetohydrazide (4d)

White solid; Yield: 82%; M.p:90-92 ^{o}C;

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.76 (* 11.58, s, 1H), 8.22 (* 8.0, s, 1H), 7.82 (d, J = 6.8 Hz, 2H), 7.50-7.42 (m, 2H), 7.22-7.18 (m, 1H), 4.20 (* 3.78, s, 2H); ESI MS: m/z, 375 $(M+1)^{+}$;

2.1.3.5 (*E*)-N'-(4-cyanobenzylidene)-2-(2-chloro-4-fluorophenyl)acetohydrazide (4e)

Pale yellow solid; Yield: 82%; M.p:116-117 ^{o}C; IR (KBr): υ $_{max}$ 3240, 3626, 3432, 3186, 3091, 2962, 2864, 2221, 1671, 1597, 1557, 1493, 1418, 1399, 1386, 1350, 1309, 1290, 1274, 1255, 1229, 1186, 1170, 1149, 1106, 1044, 931, 894, 860, 832, 801, 688, 643, 622, 579, 553, 492 cm^{-1};

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.78 (brs, 1H), 8.24 (* 8.14, s, 1H), 7.96 (s, 4H), 7.50-7.40 (m, 2H), 7.20 (d, J = 7.0 Hz, 1H), 4.20 (*(3.78, s, 2H); ESI MS: m/z, 314 $(M-1)^{+}$;

2.1.3.6 (*E*)-N'-(4-acetamidobenzylidene)-2-(2-chloro-4-fluorophenyl)acetohydrazide (4f)

White solid; Yield: 82%; M.p:107-108 ^{o}C;

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.54 (* 11.38, s, 1H), 10.10 (s, 1H), 8.18 (* 7.90, s, 1H), 7.68-7.60 (m, 4H), 7.50-7.42 (m, 2H), 7.21-7.18 (m, 1H), 4.14 (*(3.68, s, 2H), 2.05 (s, 3H); ESI MS: m/z, 347.9 $(M+1)^{+}$;

2.1.3.7(*E*)-N'-(2,4-dimethoxybenzylidene)-2-(2-chloro-4-fluorophenyl)acetohydrazide (4g)

White solid; Yield: 84%; M.p:133-134 ^{o}C; IR (KBr): υ $_{max}$ 3432, 3197, 3069, 3053, 3011, 2966, 2940, 2917, 2896, 2839, 1658, 1613, 1600, 1559, 1503, 1493, 1460, 1439, 1418, 1397, 1366, 1343, 1316, 1288, 1274, 1244, 1211, 1187, 1173, 1161, 1124, 1068, 1042, 988, 965, 923, 899, 877, 857, 837, 808, 796, 738, 689, 679, 638, 625, 584, 513 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.46 (* 11.22, s, 1H), 8.44 (* 8.22, s, 1H), 7.80 (* 7.76, d, J = 6.8 Hz, 1H), 7.56-7.40 (m, 2H), 7.22-7.18 (m, 2H), 6.62 (s, 1H), 6.60-6.56 (m, 1H), 4.16 (*3.66, s, 2H), 3.82 (s, 3H), 3.80 (* 3.78, s, 3H); ESI MS: m/z, 350.9 $(M+1)^{+}$;

2.1.3.8(*E*)-N'-(3,4-dimethoxybenzylidene)-2-(2-chloro-4-fluorophenyl)acetohydrazide(4h)

White solid; Yield: 86%; M.p:129-130 ^{o}C;

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.48 (* 11.38, s, 1H), 8.18 (* 7.98, s, 1H), 7.58-7.40 (m, 2H), 7.30 (d, J = 7.2 Hz, 1H), 7.22-7.16 (m, 2H), 7.0 (d, J = 7.2 Hz, 1H), 4.18 (*3.76, s, 2H), 3.80 (s, 6H); ESI MS: m/z, 350.9 $(M-1)^+$;

2.1.3.9(*E*)-N'-(5-bromo-2-hydroxybenzylidene)-2-(2-chloro-4-fluorophenyl) acetohydrazide (4i)

Pale yellow solid; Yield: 84%; M.p:88-89 ^oC;

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.98 (* 11.58, s, 1H), 11.16 (* 10.38, s, 1H), 8.42 (* 8.22, s, 1H), 7.80 (d, J = 7.6 Hz, 1H), 7.56-7.40 (m, 3H), 7.20 (d, J = 6.6 Hz, 1H), 6.82 (d, J = 6.4 Hz, 1H), 4.18 (*3.78, s, 2H); ESI MS: m/z, 384.9 $(M-1)^+$;

2.1.3.10 (*E*)-N'-(3,4,5-trimethoxybenzylidene)-2-(2-chloro-4-fluorophenyl) acetohydrazide (4j)

White solid; Yield: 86%; M.p:136-137 ^oC;

^{1}H NMR (500 MHz, DMSO-d_6): δ 11.60 (* 11.50, s, 1H), 8.18 (* 7.90, s, 1H), 7.50-7.42 (m, 2H), 7.22-7.16 (m, 1H), 6.98 (s, 2H), 4.18 (*3.80, s, 2H), 3.80 (s, 6H), 3.70 (s, 3H); ESI MS: m/z, 381.9 $(M+1)^+$;

2.1.3.11 (*E*)-N'-(4-hydroxy-3-methoxybenzylidene)-2-(2-chloro-4-fluorophenyl) acetohydrazide (4k)

White solid; Yield: 90%; M.p:111-112 ^oC; IR (KBr): υ_{max} 3179, 3095, 2972, 2936, 2845, 1655, 1604, 1585, 1520, 1491, 1461, 1400, 1351, 1302, 1273, 1257, 1243, 1215, 1194, 1179, 1168, 1119, 1036, 957, 944, 929, 911, 886, 853, 828, 798, 782, 702, 688, 669, 660, 617, 584, 571, 485 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.42 (* 11.32, s, 1H), 9.50 (brs, 1H), 8.16 (* 7.94, s, 1H), 7.52-7.16 (m, 1H), 7.12 (d, J = 6.4 Hz, 1H), 6.80 (d, J = 6.4 Hz, 1H), 4.18 (*3.66, s, 2H), 3.80 (s, 3H); ESI MS: m/z, 336.9 $(M-1)^+$;

2.2 ANTIBACTERIAL AND ANTIFUNGAL BIOASSAY

The antibacterial activity of all the synthesized compounds (**4a-k**) were examined against different Gram-positive (*Staphylococcus.pyogens* and *Staphylococcus aureus)* and Gram-negative (*Escherichia coli* and *Pseudomonas .aeruginosa*) organisms by measuring zone of inhibition. The antibacterial activity was performed by Agar diffusion method at the concentration level of 100µg/mL. Chloramphenicol was used as standard drug at a concentration of 100µg/mL. Nutrient agar was used as culture media and DMSO was used as solvent control.[16-18] The results of the antibacterial activity are shown in Table 1.

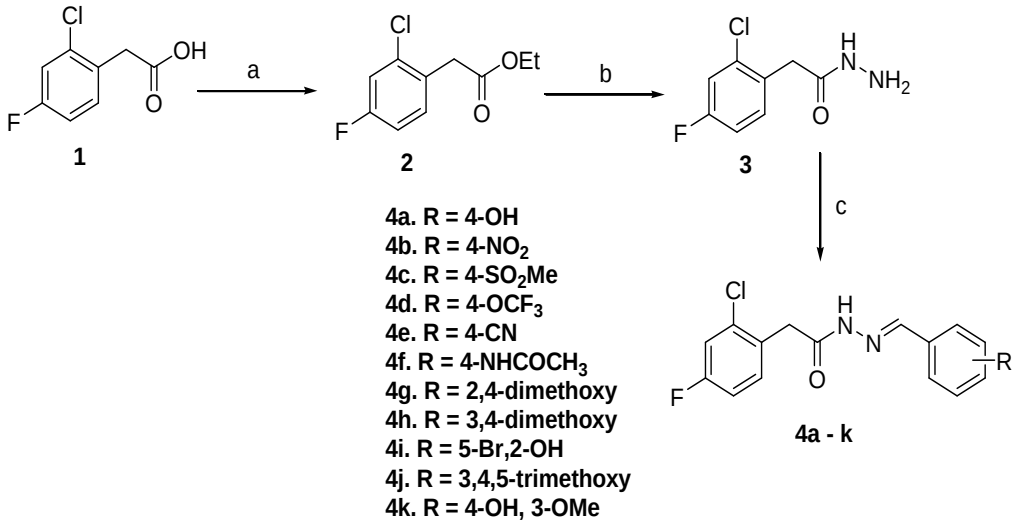
The antifungal activity of all the synthesized compounds (**4a-k**) were examined against *Aspergillus niger* and *Candida albicans* by measuring zone of inhibition. The antifungal activity was performed by Agar diffusion method at the concentration level of 100µg/mL. Nystatin was used as standard drug at a concentration of 100µg/mL. Sabouraud dextrose agar was used as culture media and DMF was used as solvent control.[19] The results of the antifungal activity are shown in Table 1.

3.0 RESULTS AND DISCUSSION

3.1 Chemistry

The targeted (*E*)-N'-(3,4,5-trimethoxybenzylidene)-2-(2-chloro-4-fluorophenyl) acetohydrazide derivatives **4a-k** were prepared in a three-step sequence as shown in **Scheme-1**. Initially, 2-(2-chloro-4-fluorophenyl)acetic acid **1** was converted to corresponding ethyl 2-(2-chloro-4-fluorophenyl)acetate **2** in presence of con H_2SO_4 (catalytic amount) in ethanol. The ethylester **2** was further treated with hydrazine hydrate to 2-(2-chloro-4-fluorophenyl)acetohydrazide. Acetohydrazides **3a-k** was reacted with various banzaldehydes **a-k** in ethanol at reflux for 1h to yield the titled compounds (**4a-k**), respectively in quantitative yield.

The synthesized acetohydrazide-hydrazone derivatives **4a-4k** was characterized by ^{1}H NMR, mass and IR spectral data. It is observed that ^{1}H NMR spectra's of these compounds were found to exist as a mixture of two rotameric forms in solution[20, 21] e.g. antiperiplanar (*ap*) and synperiplanar (*sp*). As an example, the ^{1}H NMR spectra of the compound **4d** is described here, the broad singlets at 11.76 (* 11.58 ppm) and 8.22 ppm (* 8.00 ppm) corresponds to the protons representing to –CO-**NH**- and -**HC**=N- groups respectively and the proton signal at 4.20 (* 3.78 ppm) corresponds to the –**CH_2**- proton. The doublets appearing at 7.82 and 7.40 ppm represent the para -OCF_3-phenyl ring. The protons resonating as multiplets in region 7.50-7.42 ppm (two proton integration) and 7.22-7.18 ppm (one proton integration) corresponds to 2-chloro-4-fluoro phenyl ring. Similarly, the ^{1}H NMR spectra's of the remaining aceto-hydrazide-hydrones is in concurrence with the above description. The mass spectra of compounds showed (M+1) peaks, in agreement with their molecular formula.



Scheme 1: Synthesis of novel acetohydrazide-hydrazone derivatives 4a-k

Reaction conditions: a) conc. H_2SO_4, ethanol, reflux, 5h; b) hydrazine-hydrate, ethanol, reflux, 8h; d) benzaldehydes **a-k**, ethanol, reflux, 1h.

3.2 ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

The results of the antibacterial activity data of acetohydrazones **4a-k** were compared with reference to the standard drug Chloramphenicol (for bacterial study) and Nystatin (for fungal study) against the bacterial and fungal pathogens *viz., Staphylococcus.pyogens, Staphylococcus aureus, Escherichia coli, Pseudomonas .aeruginosa, Aspergillus niger* and *Candida albicans* respectively. From table 1, it is observed that in case of *E.coli* and *P.aeruginosa*: compounds **4a, 4c, 4d, 4j** and **4k** exhibited good antibacterial activity with zone of inhibition 17-23mm (with reference to zone of inhibition of chloramphenicol; 19-23 mm) and the remaining compounds showed moderate antibacterial activity. Even in case of *S.pyogens* and *S.aureus* these compounds responded in a similar manner. In case of fungal pathogens, acetohydrazones **4a-k** displayed weak antifungal activity.

Table 1: Results of Antibacterial and Antifungal activity of Compounds 4a-k

Compound no.	*Gram negative bacteria*		*Gram positive bacteria*		*Fungi*	
	E. coli	*P.aeruginosa*	*S.pyogens*	*S.aureus*	*A. niger*	*C. albicans*
	Zone of inhibition expressed in mm					
4a	22	19	19	18	13	11
4b	12	11	11	11	11	10
4c	19	17	18	17	9	9
4d	23	19	20	20	10	12
4e	17	16	15	15	10	11
4f	16	14	16	14	9	10

4g	14	12	13	12	11	11
4h	15	12	14	13	10	10
4i	14	10	11	10	10	9
4j	20	18	18	18	12	10
4k	22	17	16	17	11	9
Chloramphenicol	23	19	20	20		
Nystatin					29	25

Standard drug concentration: 100µg/mL; "--_ NO ACTIVITY

4. CONCLUSION

In summary we have synthesized some new acetohydrazide-hydrazone derivatives **4a-k** from 2-(2-chloro-4-fluorophenyl)acetohydrazide **3** which was in turn prepared from commercially available 2-(2-chloro-4-fluorophenyl)acetic acid **1**. These compounds were tested for antibacterial and antifungal activity; the results revealed that most of the compounds exhibited good antibacterial activity however displayed weak antifungal activity against the standard drug candidates Chloramphenicol (for bacterial study) and Nystatin (for fungal study).

5. ACKNOWLEDGEMENT

One of the authors (BK), is thankful to Dr. B. Ram, Green Evolution Laboratories for helpful suggestions.

6.0 CONFLITCT OF INTEREST

"The author(s) declare(s) that there is no conflict of interest regarding publication of this article".

7.0 REFERENCE

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