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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME NOVEL HYDRAZONE DERIVATIVES OF ANTHRANILIC ACID

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ABSTRACT

This paper describes the synthesis, characterization and antibacterial evaluation of some new N acylhydrazone(NAH) compounds **5a-j,** (*E*)-N'-(substituted-benzylidene)-2-(benzamido)benzohydrazides, derived by the condensation of different aldehydes with acid hydrazide of N-benzylanthranilic acid **4.** The structures of the newly synthesized compounds were ascertained on the basis of ^{1}H NMR, mass and IR data. All the compounds have been evaluated for their antibacterial activity against (i) *Staphylococcus aureus* (MTCC 96), *(ii) Streptococcus pyogenes* (MTCC 442), *(iii) Escherichia coli* (MTCC 443) and *(iv) Pseudomonas aeruginosa* (MTCC 424) by agar well diffusion method. Most of the compounds showed good to moderate activity. Compounds **5b**, **5c**, **5g** and **5j** exhibited good antibacterial activity against the tested bacterial strains.

KEYWORDS: Anthranilic acid**,** Hydrazones, Synthesis, Antibacterial activity, Agar diffusion.

1.0 INTRODUCTION

Anthranilic acid is a constituent of many bioactive compounds that exhibits a variety of biological activities. In particular, the nucleus of anthranilic acid is the biochemical precursor to the amino acid and its derivatives as well as to the constituents of quite a few alkaloids. The biological activities of this essential moiety have been well reported. Some of them like mefenamic acid and meclofenamates, both *N*-phenyl anthranilic acid derivatives, have been used as anti-inflammatory agents[1]. Anthranilic acid is a good pharmacophore that is recurrently used in drug discovery programs[2, 3]. Anthranilic acid analogs also exhibit good selectivity against cytochrome P450 subtypes CYP2C8 and CYP2C9[4]. Among the wide variety of chemical structures used in the development of new anticancer drugs, a series of novel N-(2-pyridin-4-yl)anthranilic acid derivatives were shown to possess potent *in vitro* antiproliferative activity against human tumor cells, exhibiting preventive or inhibitory activity on account of their action through different biological mechanisms[5,6]. The *in vivo* and pharmacokinetic data provided convincing evidence that a series of N-phenyl anthranilic acid analogs could potentially generate clinical candidates for Alzheimer's disease therapy[7]. In recent years, anthranilic acid derivatives were found to exhibit antitumaral activity[8] and as a potent partially nuclear farnesoid X receptor (FXR) agonists [9] and antihepatitis C virus therapeutic agents by inhibiting hepatitis C polymerase(NS5B)[10]. The need of new anti-microbial agents is justified because more microorganisms are being resistant to the present drugs available in the market. Worldwide researchers are trying to synthesize new drugs with better pharmacokinetic and pharmacodynamic properties with less adverse effects. Hydrazone nucleus is found in natural and synthetic products of biological interest. Literature studies revealed that hydrazones and various substituted hydrazones are associated with a broad spectrum of biological activities such as antimicrobial activities[11],anti-tumor activity[12], antimycobacterial activity[13], antitubercular activity[14], anti-inflammatory[15] and anti-platelet activity[16]. Prompted by the significance of anthranilic acid in biological systems, an attempt was made to design and synthesize some new hydrazone derivatives of anthranilic acid and evaluate them for their potential antibacterial activities. Hydrazone derivatives of anthranilic acid **5a-j** were synthesized successfully with good yield and their structures were confirmed by the spectroscopic techniques such as ^{1}H NMR, mass and FTIR data.

2.0 MATERIALS AND METHODS

Chemicals and solvents were purchased from Sigma-Aldrich and Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E. Merck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellets with Perkin-Elmer Spectrum GX FTIR instrument and only diagnostic and/or intense peaks were reported. ^{1}H NMR spectra were recorded in DMSO- d_6 with Varian Mercury plus 400 MHz instrument. Signals due to the residual protonated solvent (^{1}H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as internal standard. The ^1H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity was indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the list of coupling constants (*J*) correspond to the order of multiplicity assignment. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under nitrogen atmosphere.

2.1 EXPERIMENTAL

2.1.1 Preparation of ethyl 2-aminobenzoate (2)

To a stirred solution of 2-amino-benzoicacid (1.5g, 10.94 mmol) in ethanol (15.0 mL) was added conc. H_2SO_4 (0.2 mL) and refluxed for 10 h. After completion of the reaction (monitored by T.L.C), the reaction mixture was diluted with water and extracted with ethyl acetate (25 mL).The organic layer was washed with aqueous saturated $NaHCO_3$ solution, water and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to obtain compound **2** as a pale yellow liquid. Yield: 1.5g, 83%. The crude compound was utilized in the next step without further purification.

2.1.2 Preparation of ethyl 2-(benzamido)benzoate (3)

To a solution of ethyl 2-aminobenzoate **2** (1.5g, 9.08 mmol) in dichloromethane, cooled to 10^{o}C, was added triethyl amine (1.10g, 10.90 mmol) followed by drop wise addition of benzoyl chloride (1.3g, 9.25 mmol). The reaction mixture was stirred at room temperature for 3h and then diluted with water followed by ethyl acetate (50 mL). The organic layer was washed with water and brine solution, dried over Na_2SO_4, filtered and evaporated to obtain ethyl 2-(benzamido)benzoate **3** as a pale yellow viscous liquid**;** Yield: 1.5g, 60%; IR (KBr): υ_{max}3245 (NH str), 1687 (C=O str, ester), 1671 (C=O str, amide), 1531 (NH def) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H, NH), 8.57 (d, *J* = 8.8 Hz, 1H, Ar), 8.03 (dd, *J* = 8.0, 1.4 Hz, 1H, Ar), 8.01 – 7.94 (m, 2H, Ar), 7.74 – 7.54 (m, 4H, Ar), 7.26 (t, *J* = 7.7 Hz, 1H), 4.36 (q, *J* = 7.1 Hz, 2H, CH_2), 1.32 (t, *J* = 7.1 Hz, 3H, CH_3); ESI MS: *m/z* 269.8 $(M+H)^+$.

2.1.3 Preparation of 2-(benzamido)benzohydrazide (4)

To a stirred solution of compound **3** (1.5g, 5.60 mmol) in 15ml ethanol, hydrazine hydrate (2.29 g, 45.80 mmol) was added and refluxed for 6 h. The reaction mixture was cooled to room temperature and the precipitated solid was filtered, washed with pet-ether and dried under vacuum to obtain 2-(benzamido)benzohydrazide**4 as** white solid. Yield: 1.10g, 80%;

M.p: 185-186 ^oC; IR (KBr): υ_{max}3317 (NH str), 1649 (C=O str), 1634 (C=O str), 1537 (NH def) cm^{-1};^1H NMR (400 MHz, DMSO-d_6) δ 12.57 (s, 1H, NH), 10.21 (s, 1H, NH), 8.67 (d, *J* = 8.4 Hz, 1Hr, Ar), 7.97 (d, *J* = 7.3 Hz, 2H, Ar), 7.79 (d, *J* = 7.8 Hz, 1H, Ar), 7.61 (m, 4H, Ar), 7.18 (t, *J* = 7.8 Hz, 1H, Ar), 4.69 (s, 2H, NH_2); ESI MS: *m/z* 255.8 $(M+H)^+$.

2.1.4 General experimental procedure for the preparation of hydrazone derivatives 5a-j

To a stirred solution of compound **4** (100 mg, 0.392mmol) in 1.0 mL ethanol, was added the corresponding aromatic aldehydes **a-j** (0.392 mmol) and refluxed for 1 h. The reaction mixture was cooled to room temperature and the solid precipitated was dried to obtain the hydrazone derivatives **5a-j**. Yields of the products varied between 85 to 95%.

2.1.4.1 Spectral characteristics of (*E*)-N'-(4-bromobenzylidene)-2-benzamido)benzohydrazide (5a)

White solid; Yield: 90%; M.p: 92-93^oC ; IR (KBr): υ_{max}3328 (NH str), 1652 (C=O &C=N str), 1530 (NH def) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H, NH), 11.89 (s, 1H, NH), 8.56 (d, *J* = 8.3 Hz, 1H, Ar), 8.44 (s, 1H, N=CH), 7.96 (d, *J* = 7.2 Hz, 2H, Ar), 7.91 (d, *J* = 7.6 Hz, 1H, Ar), 7.76- 7.57 (m, 8H, Ar), 7.30 (t, *J* = 7.6 Hz, 1H, Ar); ESI MS: *m/z* 421.8 $(M+H)^+$.

2.1.4.2 Spectral characteristics of (*E*)-N'-(4-bromobenzylidene)-2-benzamido) benzohydrazide (5a)

White solid; Yield: 88%; M.p: 112-113^oC ; ^1H NMR (400 MHz, DMSO-d_6) δ 12.16 (s, 1H, NH), 11.93 (s, 1H, NH), 8.57 (d, *J* = 8.5 Hz, 1H, Ar), 8.46 (s, 1H, N=CH), 7.97 (d, *J* = 7.4 Hz, 2H, Ar), 7.91 (d, *J* = 7.6 Hz, 1H, Ar), 7.83 (t, J = 7.0 Hz, 2H, Ar), 7.69-7.56 (m, 4H, Ar), 7.34- 7.27 (m, 3H, Ar);ESI MS: *m/z* 361.9 $(M +H)^+$;

2.1.4.3 Spectral characteristics of (*E*)-N'-(4-(trifluoromethoxy)benzylidene)-2-(benzamido)benzohydrazide (5c)

White solid; Yield: 90%; M.p: 88-89^oC; IR (KBr): υ_{max} 3323 (NH str), 1660 (C=O str), 1650 (C=N str), 1525 (NH def) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H, NH), 11.89 (s, 1H, NH), 8.56 (d, *J* = 8.3 Hz, 1H, Ar), 8.49 (s, 1H, N=CH), 7.97 (d, *J* = 7.6 Hz, 2H, Ar), 7.94 – 7.82 (m, 3H, Ar), 7.71 – 7.56 (m, 4H, Ar),7.48 (d, *J* = 8.2 Hz, 2H, Ar), 7.30 (t, *J* = 7.6 Hz, 1H, Ar); ESI MS: *m/z* 427.9 $(M+H)^+$.

2.1.4.4 Spectral characteristics of (*E*)-N'-(4-(dimethylamino)benzylidene)-2-(benzamido)benzohydrazide (5d)

White solid; Yield: 88%; M.p: 76-77^{o}C; IR (KBr): υ_{max}3192 (NH str), 1663 (C=O str), 1645 (C=N str),1523 (NH def) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.11 (s, 1H, NH), 11.86 (s, 1H, NH), 8.61 (d, *J* = 8.3 Hz, 1H, Ar), 8.32 (s, 1H, N=CH), 7.97 (d, *J* = 7.2 Hz, 2H, Ar), 7.90 (d, *J* = 7.6Hz, 1H, Ar), 7.70 – 7.59 (m, 4H, Ar), 7.57 (d, *J* = 8.7 Hz, 2H, Ar), 7.27 (t, *J* = 7.6 Hz, 1H, Ar), 6.77 (d, *J* = 8.7 Hz, 2H Ar), 2.99 (s, 6H, NCH_3); ESI MS: *m/z* 386.9 $(M+H)^+$.

2.1.4.5 Spectral characteristics of (*E*)-N'-(4-nitrobenzylidene)-2-(benzamido)benzohydrazide (5e)

White solid; Yield: 96%; M.p: 119-120^{o}C; IR (KBr): υ_{max} 3263 (NH str), 1667 (C=O str), 1651 (C=N str), 1519 (NH def),1518, 1340 (NO_2) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.42 (s, 1H, NH), 11.79 (s, 1H, NH), 8.56 (s, 1H, N=CH), 8.53 (d, *J* = 8.3 Hz, 1H, Ar), 8.33 (d, *J* = 8.6 Hz, 2H, Ar), 8.03 (d, *J* = 8.6 Hz, 2H, Ar), 7.97 (d, *J* = 7.1 Hz, 2H, Ar), 7.92 (d, *J* = 7.6 Hz, 1H, Ar), 7.71 – 7.54 (m, 4H, Ar), 7.32 (t, *J* = 7.6 Hz, 1H, Ar); ESI MS: *m/z* 388.9 $(M+H)^+$.

2.1.4.6 Spectral characteristics of (*E*)-N'-(4-cyanobenzylidene)-2-(benzamido)benzohydrazide (5f)

White solid; Yield: 84%; M.p: 126-128^{o}C ; IR (KBr): υ_{max} 3263 (NH str), 1665 (C=O str), 1654 (C=N str), 1521 (NH def),2221 (C≡N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.36 (s, 1H, NH), 11.81 (s, 1H, NH), 8.53 (d, *J* = 8.5 Hz, 1H, Ar), 8.51 (s, 1H, N=CH), 8.02 – 7.86 (m, 7H, Ar), 7.69 – 7.55 (m, 4H, Ar), 7.31 (t, *J* = 7.5 Hz, 1H, Ar); ESI MS: *m/z* 368.9 $(M+H)^+$.

2.1.4.7 Spectral characteristics of (*E*)-N'-(4-(methylsulfonyl)benzylidene)-2-(benzamido)benzohydrazide (5g)

White solid; Yield: 86%; M.p: 132-133^{o}C ; IR (KBr): υ_{max} 3354 (NH str), 1667 (C=O str), 1650 (C=N str), 1531 (NH def) cm^{-1};^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.36 (s, 1H, NH), 11.81 (s, 1H, NH), 8.54 (s, 1H, N=CH), 8.53 (d, *J* = 8.2 Hz, 1H, Ar), 8.02 (s, 4H, Ar), 7.97 (d, *J* = 7.2 Hz, 2H, Ar), 7.92 (d, *J* = 7.6 Hz, 1H, Ar), 7.73 – 7.47 (m, 4H, Ar), 7.31 (t, *J* = 7.6 Hz, 1H, Ar), 3.27 (s, 3H, SCH_3); ESI MS: *m/z* 421.9 $(M+H)^+$.

2.1.4.8 Spectral characteristics of (*E*)-N'-(2,4-dimethoxybenzylidene)-2-(benzamido)benzohydrazide (5h)

White solid; Yield: 88%; M.p: 141-142^{o}C ; IR (KBr): υ_{max} 3246 (NH str), 1674 (C=O str), 1636 (C=Nstr), 1534 (NH def) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.12 (s, 1H, NH), 12.00 (s, 1H, NH), 8.74 (s, 1H, N=CH), 8.61 (d, *J* = 8.3 Hz, 1H, Ar), 7.97 (d, *J* = 7.4 Hz, 2H, Ar), 7.93 (d, *J* = 7.6 Hz, 1H, Ar), 7.88 – 7.81 (m, 1H, Ar), 7.70 – 7.57 (m, 4H, Ar), 7.27 (t, *J* = 7.6 Hz, 1H, Ar), 6.69 – 6.63 (m, 2H, Ar), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3); ESI MS: *m/z* 404.0 $(M+H)^+$.

2.1.4.9 Spectral characteristics of (*E*)-N'-(2,5-dimethoxybenzylidene)-2-(benzamido)benzohydrazide (5i)

White solid; Yield: 92%; M.p: 100-101^{o}C; IR (KBr): υ_{max}3204 (NH str), 1650 (C=O, C=N str),1525 (NH def) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H, NH), 11.96 (s, 1H, NH), 8.81 (s, 1H, N=CH), 8.57 (d, *J* = 8.3 Hz, 1H, Ar), 7.97 (d, *J* = 7.4 Hz, 2H, Ar), 7.93 (d, *J* = 7.6 Hz, 1H, Ar), 7.70 – 7.56 (m, 4H, Ar), 7.40 (d, *J* = 3.2 Hz, 1H, Ar), 7.28 (t, *J* = 7.6 Hz, 1H, Ar), 7.08 (d, *J* = 8.9 Hz, 1H, Ar), 7.04 (dd, *J* = 8.9, 3.2Hz, 1H, Ar), 3.83 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3); ESI MS: *m/z* 404.0 $(M+H)^+$.

2.1.4.10 Spectral characteristics of (*E*)-N'-(3,4,5-trimethoxybenzylidene)-2-(benzamido)benzohydrazide (5j)

White solid; Yield: 95%; M.p: 118-119^{o}C; IR (KBr): υ_{max} 3219 (NH str), 1665 (C=O str), 1649 (C=N str), 1513(NH def) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H, NH), 11.82 (s, 1H, NH), 8.52 (d, *J* = 8.3 Hz, 1H, Ar), 8.38 (s, 1H, N=CH), 7.96 (d, *J* = 7.6 Hz, 2H, Ar), 7.89 (d, *J* = 7.6 Hz, 1H, Ar), 7.70 – 7.56 (m, 4H, Ar), 7.30 (t, *J* = 7.6 Hz, 1H, Ar), 7.04 (s, 2H, Ar), 3.85 (s, 6H, OCH_3), 3.72 (s, 3H, OCH_3); ESI MS: *m/z* 434.0 $(M+H)^+$.

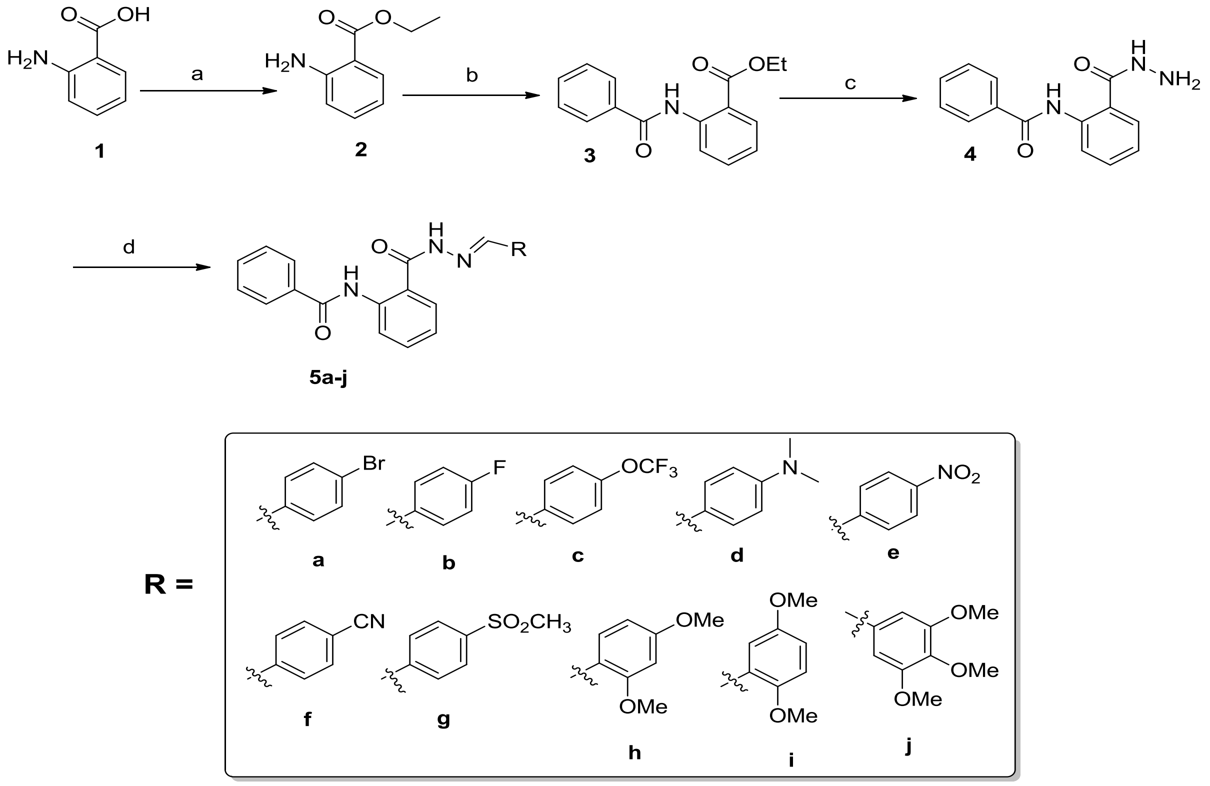
2.2 ANTIBACTERIAL BIOASSAY

The synthesized (*E*)-N'-(substituted-benzylidene)-2-(benzamido)benzohydrazides **(5a-5j)** were tested against Gram positive strains of (i) *Staphylococcus aureus*(MTCC 96) and (*ii)* *Streptococcus pyogenes*(MTCC 442) and Gram negative strains of (*iii) Escherichia coli* (MTCC 443) and *(iv) Pseudomonas aeruginosa*(MTCC 424) using agar well diffusion method according to the literature protocol[17,18]. The compounds were dissolved in dimethylsulphoxide at 50 µg mL^{-1} concentration and Ciprofloxacin was used as the reference antibacterial drug. Antibacterial activity of the compounds was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. All the samples were taken in triplicate

Table I: Antibacterial Activity of Compounds 5a-j (Concentration Used 50 µg/mL of DMSO)

Compound No	**R**	**Gram negative bacteria**		**Gram positive bacteria**	
		E.coli* *MTCC 443	***P.aeruginosa* *MTCC 424***	***S.aureus* *MTCC 96***	***S.pyogenes* *MTCC 442***
		Zone of inhibition* [b] *in mm			
5a	4-Br	**10**	**9**	**9**	**12**
5b	4-F	**22**	**22**	**21**	**19**
5c	4-OCF$_3$	**24**	**21**	**20**	**18**
5d	-N (CH$_3$)$_2$	**16**	**15**	**12**	**13**
5e	4-NO$_2$	**11**	**11**	**11**	**10**
5f	4-CN	**10**	**10**	**9**	**8**
5g	4-SO$_2$CH$_3$	**23**	**22**	**23**	**20**
5h	2,4-OMe	**16**	**17**	**17**	**14**
5i	2,5-OMe	**15**	**16**	**15**	**14**
5j	3,4,5-OMe	**24**	**23**	**20**	**19**
[a] Standard Drug	-	28	24	21	21

[a] Ciprofloxacin (50 µg/mL of DMSO); [b] Zone of inhibition - good activity: 18-24 mm; moderate activity: 12-17 mm; weak activity: < 12 mm;



Scheme 1: Synthesis of novel hydrazone derivatives 5a-j from anthranilic acid

Reaction conditions: a) conc. H_2SO_4, ethanol, reflux, 10 h; b) benzoyl chloride, triethylamine, dichloromethane, 10°C –R.T, 3h; c) hydrazine-hydrate, ethanol, reflux, 6h; d) aldehydes **a-j**, ethanol, reflux, 1h.

3.0 RESULTS AND DISCUSSION

3.1 Chemistry

Synthesis of the title compounds is outlined in scheme 1. Esterification of anthranilic acid in the presence of conc. H_2SO_4 and ethanol and refluxing for 10 h resulted in ethyl 2-aminobenzoate **2**. Amide coupling of amine **2** was accomplished by reacting with benzoyl chloride in the presence of triethylamine in dichloromethane at room temperature for 3h which gave ethyl 2-(benzamido)benzoate **3**. Hydrazinolysis of benzoate **3** in the presence of hydrazine hydrate in ethanol and reflux for 6h resulted in 2-(benzamido) benzohydrazide **4.** Condensation of compound **4** with various aldehydes, **a-j**, was done in ethanol with reflux for 1h resulted in the formation of (*E*)-N'-(substituted-benzylidene)-2-(benzamido)benzohydrazides **5a-j.** The structures of newly synthesized benzohydrazide derivatives **5a-j** were ascertained by spectroscopic techniques,^{1}H NMR, mass and IR spectral data.

In the ^{1}H NMR spectra of hydrazones**5a-j**, represented the characteristic signals in the region 12.57-12.11 ppm, 12.00-10.21 ppm and 8.81- 8.32 ppm corresponding to hydrazone NH, amide NH and –N=CH- respectively and the aromatic protons, appeared in the region 6.77-8.67 ppm, while the aliphatic protons appeared in the expected region. The mass spectra of the compounds showed (M+1) peaks and are in agreement with their molecular formulae. The IR spectra of the compounds **5a-j** represented the characteristic peaks (distinct stretching peaks given in the experimental section) that comply with the desired functional group in the structure.

3.2BIOLOGY

The newly synthesized compounds **5a-j**were evaluated for *in-vitro* antimicrobial activity studies against microorganisms and the results are discussed. The bioactivity studies were carried out against the bacteria, *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa*.

3.2.1Antibacterial activity of anthranilic acid hydrazones 5a-j (agar diffusion assay)

The agar diffusion method [17, 18] was used for the determination of antibacterial activity of synthesized anthranillic acid hydrazones against microorganism listed above. Compounds **5b** (R = 4-F), **5c** (R = 4-OCF$_3$), **5g** (4-SO$_2$CH$_3$) and **5j** (3,4,5-OMe) exhibited good antibacterial activity against *E.coli* and *P.aeruginosa* with inhibition zone 21-24 mm while the compounds **5d** (N(CH$_3$)$_2$), **5h** (R = 2,4-OMe) and **5i** (R = 2,5-OMe) showed moderate activity with zone of inhibition 15-17 mm. These compounds exhibited similar pattern of antimicrobial activity against *S.aureus* and *S.pyogenes* with variation in zone of inhibition i.e. 18-23 mm for good activity, 12-17 mm for moderate activity. The remaining compounds such as **5a** (R=4-Br), **5e** (R = 4-NO$_2$) and **5f** (R= 4-CN) were observed to be less active towards all the above tested pathogens.

4. CONCLUSION

Hydrazone derivatives 5a-j were prepared in four steps from commercially available anthranilic acid and evaluated for antibacterial activity. The results of the antibacterial activity screening revealed that compounds **5b** (R = 4-F), **5c** (R = 4-OCF$_3$), **5g** (4-SO$_2$CH$_3$) and **5j** (3,4,5-OMe) exhibited good antibacterial activity against the tested bacterial strains.

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6.0CONFLITCT OF INTEREST

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

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