WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 914-926. Research Article ISSN 2277– 7105

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF BENZOHYDRAZIDE DERIVATIVES LINKING THIENO[3,2-c]PYRIDINE NUCLEUS

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Article Received on
14 September 2017,
Revised on 04 October 2017,
Accepted on 24 October 2017
DOI: 10.20959/wjpr201714-10005

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ABSTRACT

The present paper describes the synthesis and antibacterial activity of thieno [3, 2-c] pyridine-hydrazide–hydrazone derivatives (**9a-j**) from readily accessible starting material Thiophene aldehyde. All the newly synthesized hydrazone derivatives are characterized by ^{1}H NMR, mass and IR data. These compounds were further evaluated for antibacterial activity against Gram-positive and Gram negative bacteria. Most of the compounds showed promising anti-bacterial activity.

KEYWORDS: Hydrazones, Thieno[3,2-c]pyridine, 2-Thiophenealdehyde, Antibacterial activity, Synthesis.

1. INTRODUCTION

Hydrazone compounds constitute an important class for new drug development in order to discover an effective compound against multidrug resistant microbial infection. Hydrazide-hydrazones have been demonstrated to possess anticonvulsant[1], antidepressant[2], anti-inflammatory[3], anti malarial[4], anti mycobacterial[5], anticancer[6], and antibacterial[7-10] activities. The treatment of bacterial infections remains a challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Therefore, there is an urgent need for development of new antibacterial agents with divergent and unique structure and with a mechanism of action possibly different from that of existing antimicrobial agents.[10,11]

The search for pharmacologically active substances has led to the investigation of isoquinoline isosters (furopyridines, thienopyridines) in which the benzene ring is replaced by a thiophene nucleus. In addition, thiophenes with fused six membered hetrocyclic ring are currently attracting special interest. The thieno[3,2-c]pyridine derivatives are emerging as a useful pharmacophore in several therapeutic areas such as, as protease kinase inhibitor[12], antipsychotic activity[13], antithrombin activity.[14] In addition to the above biological activities, in recent years some of the thieno[3,2-c] derivatives exhibited fluorescence properties.[15,16]

Encouraged by these interesting biological activities associated with hydrazone derivatives and thieno[3,2-c]pyridine derivatives, we report here in the synthesis, characterization and antibacterial activity of thieno[3,2-c]pyridine-hydrazide–hydrazone derivatives (9a-9j) derived from N-(2-formylthieno[3,2-c]pyridin-4-yl) benzamide 7 in a few high yielding steps from commercially available 2-Thiophenaldehyde (Scheme 1). The synthesized targets were screened for their antibacterial activity against *Escheria.Coli*, *Pseudomonas.aeruginosa*, *Staphylococcus. aureus* and *Streptococus .pyogenes*, while using Norfloxacin as the reference drug candidate.

2. 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 eluting solvents are indicated in the procedures. Merck silica gel 60 (230-400 mesh) was used for flash column chromatography. Melting point (mp) determinations were performed by using Mel-temp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Unity instrument at room temperature at 400MHz. Chemical shifts are reported in δ parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent 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 (*E*)-3-(thiophen-2-yl)acrylic acid (1)

To a mixture of 2-thiophene aldehyde (5 g, 52.03 mmol), malonic acid (5.95 g, 57.23 mmol), TBAB (26.0 mmol), K_2CO_3 (26.0 mmol) and distilled water (25 mL) was irradiated in a microwave oven at 900 W for 5 min at 100^{o}C. After complete conversion as indicated by TLC, the reaction mass was poured into the ice cold water (50 mL) and the precipitated solid was filtered and dried under vacuum to afford compound **1. Pale brown solid,** Yield 89%;

M.p: 144-145^{o}C; IR (KBr): υ_{max} 1691 (-C=O), 1673 (-CH=CH-CO, α,β-unsaturated str.), 1410 (C=C str.) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.90 (d, 1H, -CH=CH-C=O, J = 15.6 Hz), 7.44 (d, 1H, thiophene-H, J = 5.2 Hz), 7.32 (d, 1H, thiophene-H, J = 3.6 Hz), 7.08 (dd,1H, thiophene-H, J = 5.2, 1.4 Hz), 6.26 (d, 1H, -CH=CH-C=O, J = 15.6 Hz); EI MS: m/z (rel.abund.%) 153.2 (M^-, 100).

2.1.2 Preparation of (*E*)-1-azido-3-(thiophen-2-yl) prop-2-en-1-one (2)

To a solution of cyanuric chloride (2.27g, 5 mmol) in dichloromethane (60 mL), N-methylmorpholine (4.05 mL, 36.90 mmol) was added at 0-5^{o}C with continuous stirring. A white suspension was formed to which a solution of the acrylic acid **1** (5.0 g, 36.90 mmol) in dichloromethane (10 mL) was added and the stirring was continued for 3 h. The mixture was filtered through and to this filtrate, NaN_3 (2.40 g, 36.90 mmol) added and the stirring was continued for 3 h at room temperature. After completion of the reaction (TLC), the mixture was washed with a saturated solution of $NaHCO_3$ (3 x 10 mL) and then with water (3 x 10 mL). The organic layer was dried with anhydrous Na_2SO_4, passed through a short silica-gel column, and the solvent removed under reduced pressure to afford **2. Off-white solid;** Yield 78%; M.p: 80-81^{o}C; IR (KBr): υ_{max} 2140 (-N_3), 1676 (-CH=CH-CO, α,β-unsaturated str.), 1415 (C=C str.) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.86 (d, 1H, -CH=CH-C=O, J = 15.6 Hz), 7.46 (d, 1H, thiophene-H, J = 4.8 Hz), 7.32 (d, 1H, thiophene-H, J = 3.6 Hz), 7.08 (dd,1H, thiophene-H, J = 4.4, 1.2Hz), 6.26 (d, 1H, -CH=CH-C=O, J = 15.6 Hz); EI MS: m/z (rel.abund.%) 182.3 (M^-, 100).

2.1.3 Preparation of thieno[3,2-c]pyridin-4(5H)-one (3)

To a solution of tributyl amine (4.0 mL, 16.86 mmol) in diphenyl ether (30 mL) heated at 230°C was added, a pre-mixed solution of azide **2** (5 g, 30.67 mmol) in dichloromethane (15 mL) over a period of 30 min. The reaction mixture was continued to stir at the same temperature for another 30 min. The reaction mixture was cooled to room temperature and diluted with 150 mL of hexane. After stirring for 15 min, the precipitated solid was filtered, washed with hexane (2 x 100 mL) and dried under vacuum to afford 3. Pale yellow solid; Yield 80%; M.p: 208-209^{o}C; IR (KBr): υ_{max} 1647 (-C=ONH str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 12.17 (br.s, 1H, NH), 7.68 (d, 1H, -thiophene-H, J = 5.6 Hz), 7.34 (d, 1H, thiophene-H, J = 5.4 Hz), 7.28 (d, 1H, pyridone-H, J = 8.8 Hz), 6.80 (d, 1H, pyridone-H, J = 7.6 Hz); EI MS: m/z (rel.abund.%) 152.2 (M^-, 100).

2.1.4. Preparation of 4-chlorothieno[3,2-c]pyridine (4)

To a solution of trichloroisocyanuric acid (8.60 g, 37.0 mmol) in toluene (25 mL) was added triphenylphosphine (29.11 g, 111 mmol), at 0-5^{o}C with continuous stirring for 15 min. To the above reaction mixture, compound **3** (56.95 mmol) was added and refluxed for 5.5 h. After completion of the reaction (TLC), the solvent was evaporated to obtain the crude compound. The crude compound was purified by flash column chromatography using 3-5% of MeOH/$CHCl_3$ as an eluent to afford **4. Yellow solid;** Yield 86%; M.p: 101^{o}C; IR (KBr): υ_{max} 1647 (-C=ONH str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.24 (d, 1H, -pyridine-H, J = 5.2 Hz), 7.74 (d, 1H, pyridine-H, J = 6.0 Hz), 7.56 (d, 2H, thiophene-H, J = 18.0 Hz); EI MS: m/z (rel. abund. %) 170.2 (M^+, 100).

2.1.5 Preparation of thieno[3,2-c]pyridin-4-amine (5)

To a stainless steel reactor was added compound **4** (5 g, 29.41 mmol), followed by 1, 4-dioxane (30 mL) and 28% aqueous ammonia (50 mL). The mixture was heated to 150 ^{o}C and developed about 250 psi of pressure. After 17 h, the reaction mixture was cooled to rt and concentrated *in vacuum*. The residue was dissolved in chloroform, washed with brine solution, dried over *anhydrous* Na_2SO_4 and concentrated. The crude compound was purified by flash column chromatography using 3-5% of MeOH/$CHCl_3$ as an eluent to afford amine **5. Brown solid;** Yield 44%; M.p: 96-97^{o}C; IR (KBr): υ_{max} 1647 (-C=ONH str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.15 (br.s, 1H, -NH_2), 7.90 (d, 1H, -pyridine-H, J = 5.6 Hz), 7.36 (d, 1H, pyridine-H, J = 6.0 Hz), 7.26 (d, 1H, thiophene-H, J = 6.0 Hz), 7.20 (d, thiophene-H, J = 6.0 Hz); EI MS: m/z (rel.abund.%) 151.2 (M^+, 100).

2.1.6 Preparation of N-(thieno[3,2-c]pyridin-4-yl)benzamide (6)

To a stirred solution of thieno[3, 2-c] pyridin-4-amine (2.23 mmol) in DMF (25 vol) and triethyl amine (4.46 mmol) was added benzoic acid (2.23 mmol) followed by HOBT (2.68 mmol) and HBTU (2.68 mmol) and stirred at r.t for 12 h to 16 h. Upon completion, the reaction mixture was concentrated and the residue was extracted with EtOAc. The combined organic layer was washed with water, brine solution, dried and concentrated to afford the crude amide compounds. The crude products are either recrystallized or purified by column chromatography to afford the title compounds **6.** White solid; Yield: 75%; M.p: 125-126 °C; IR (KBr): υ_{max} 3248, 3121, 1582, 1532, 1494, 1450 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.0 (s, 1H, D_2O exchangeable), 8.28 (d, J = 8.0 Hz, 1H), 8.10 (dd, J = 2.8, 7.4 Hz, 2H), 7.98

(dd, J = 2.2, 6.8 Hz, 1H), 7.81 (dd, J = 2.6, 7.2 Hz, 1H), 7.66 - 7.50 (m, 3H), 7.34 (d, J = 7.6 Hz, 1H); EI-MS: m/z (rel. abund.%) 255.2 (M^+, 100).

2.1.7 Preparation of N-(2-formylthieno[3,2-c]pyridin-4-yl)benzamide (7)

n-Butyl lithium was added to a stirred solution of diisopropylamine (10.80 mL, 37.78 mmol) in tetrahydrofuran (20 mL) under N_2 at –78^{0}C and stirred for 20 min. To the above solution LDA solution, maintained at -78^{o}C was added a pre-mixed solution of compound **6** (4.5 g, 18.90 mmol) in tetrahydrofuran (10 mL) drop wise over a period of 30 min and stirred for 45 min. To the above reaction mixture a pre-mixed solution N, N-dimethyformamide (3.75 mL, 49.10 mmol) in tetrahydrofuran (20 mL) was added and stirred for 10 min. The reaction mixture was allowed to attain room temperature and was poured into an ice cold 1N aqueous hydrochloric acid solution (100 mL), the precipitate solid was isolated by filtration and dried.

Pale Yellow solid; Yield: 70%; M.p: 143-144°C; IR (KBr): υ_{max} 3506, 3414, 3229, 2923, 1678, 1651, 1588, 1513, 1485, 1442, 1313 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ11.20 (br.s, 1H), 10.20 (s, 1H), 8.44 (d, J = 6.6 Hz, 1H), 8.38 (d, J = 6.0 Hz, 1H), 8.12 (d, J = 6.8 Hz, 2H), 8.01 (d, J = 6.8 Hz, 1H), 7.68 - 7.54 (m, 3H); EI-MS: m/z (rel.abund.%) 283.3 (M^+, 100).

2.1.8 General Experimental Procedure for the Synthesis of Thiophen [3, 2-c] pyridine Hydrazide-hydrazone Derivatives (9a-9j)

To a stirred solution of compound **7** (0.30 mmol) in ethanol was added corresponding benzohydrazides **8a-8i** (0.3 mmol) and refluxed for 30 min. The precipitated solids were filtered and washed with cold ethanol and dried to obtain the compounds **9a-9j** in 80-88% yield.

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)benzohydrazide (9a)

Yellow solid; Yield: 82%; M.p: 126-128°C; IR (KBr): υ_{max} 3260 (-NH str), 1655 (-C=O str) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.13 (s, 1H), 11.06 (s, 1H), 8.77 (s, 1H), 8.35 (d, J = 5.6 Hz, 1H), 8.10 (d, J = 7.6 Hz, 2H), 7.98 (d, J = 5.2 Hz, 1H), 7.91 (d, J = 7.6 Hz, 2H), 7.64-7.52 (m, 7H); LC-MS: m/z, 401.05 (M+1).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-4-chlorobenzohydrazide (9b)

Pale Yellow solid; Yield: 86%; M.p: 119-120°C; IR (KBr): υ_{max} 3194 (-NH str), 1645 (-C=O str) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.18 (s, 1H), 11.06 (s, 1H), 8.75 (s, 1H), 8.35 (d, J = 5.2 Hz, 1H), 8.10 (d, J = 7.2 Hz, 2H), 7.98 (d, J = 5.2 Hz, 1H), 7.92 (d, J = 7.2 Hz, 2H), 7.74 (s, 1H), 7.66-7.55 (m, 5H); LC-MS: m/z, 435.36 (M+1).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-4-bromobenzohydrazide (9c) Pale yellow solid; Yield: 88%; M.p: 120-121 °C; IR (KBr): υ_{max} 3212 (-NH str), 1643 (-C=O str) cm^{-1};^1H NMR (400 MHz, DMSO-d_6): δ 12.20 (brs, 1H), 11.60 (brs, 1H), 8.42 (s, 1H), 8.32 (d, J = 5.2 Hz, 1H), 8.02 (d, J = 7.6 Hz, 2H), 7.84 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H), 7.62 (d, J = 7.2 Hz, 2H), 7.54 (t, J = 8.0 Hz, 2H), 7.38 (s, 1H); LC-MS: m/z, 480.01 (M+2).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-4-methoxybenzohydrazide (9d)

Pale yellow solid; Yield: 88%; M.p: 120-121°C; IR (KBr): υ_{max} 3445 (-NH str), 1647 (-C=O str) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 12.10 (s, 1H), 11.08 (brs, 1H), 8.77 (brs, 1H), 8.34 (d, J = 5.6 Hz, 1H), 8.10 (d, J = 7.2 Hz, 2H), 7.97 (d, J = 5.2 Hz, 1H), 7.91 (d, J = 8.8 Hz, 2H), 7.70 (s, 1H), 7.64 (t, J = 7.2 Hz, 1H), 7.57 (t, J = 7.6 Hz, 2H), 7.07 (d, J = 8.8 Hz, 2H), 3.86 (s, 1H); LC-MS: m/z, 431.20 (M+2).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-3-nitrobenzohydrazide (9e) Yellow solid; Yield: 88%; M.p: 113-114°C; IR (KBr): υ_{max} 3214 (-NH str), 1656 (-C=O str) cm^{-1};^1H NMR (400 MHz, DMSO-d_6): δ 12.42 (s, 1H), 11.05 (brs, 1H), 8.78 (s, 1H), 8.73 (s, 1H), 8.46 (d, J = 7.2 Hz, 1H), 8.36 (d, J = 7.2 Hz, 2H), 8.10 (d, J = 7.2 Hz, 2H), 7.99 (t, J = 5.2 Hz, 1H), 7.85 (t, J = 8.0 Hz, 1H), 7.78 (s, 1H), 7.66-7.55 (m, 3H); LC-MS: m/z, 446.32 (M+1).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-4-hydroxybenzohydrazide (9f)Off-white solid; Yield: 80%; M.p: 92-94°C; IR (KBr): υ_{max} 3418 (-OH str), 3324 (-NH str), 1649 (-C=O str) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 11.90 (s, 1H), 11.12 (s, 1H), 10.12 (s, 1H), 8.40 (s, 1H), 8.30 (d, J = 5.6 Hz, 1H), 8.0 (d, J = 7.2 Hz, 2H), 7.80 (d, J = 8.8 Hz, 2H), 7.66-7.54 (m, 5H), 7.26 (s, 1H), 6.84 (d, J = 8.4 Hz, 2H); LC-MS: m/z, 417.20 (M+1).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-3,5-dichlorobenzohydrazide (9g)

White solid; Yield: 86%; M.p: 138-139°C; IR (KBr): υ_{max} 3236 (-NH str), 1661 (-C=O str) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 12.27 (s, 1H), 11.05 (s, 1H), 8.73 (s, 1H), 8.35 (d, J = 5.2 Hz, 1H), 8.10 (t, J = 7.2 Hz, 2H), 7.98 (d, J = 5.2 Hz, 1H), 7.92 (d, J = 7.2 Hz, 2H), 7.89 (brs, 1H), 7.77 (s, 1H), 7.66-7.55 (m, 3H); LC-MS: m/z, 470.34 (M+1).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-3,4,5-trimethoxybenzohydrazide (9h)

Off White solid; Yield: 88%; M.p: 106-108°C; IR (KBr): υ_{max} 3194 (-NH str), 1645 (-C=O str) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.79 (s, 1H), 11.04 (s, 1H), 8.81 (s, 1H), 8.35 (d, J = 5.2 Hz, 1H), 8.10 (d, J = 7.2 Hz, 2H), 7.98 (d, J = 5.2 Hz, 1H), 7.71 (s, 1H), 7.64 (t, J = 7.2 Hz, 1H),7.56 (t, J = 7.2 Hz, 2H), 7.22 (s, 2H), 3.86 (s, 6H), 3.73 (s, 3H); LC-MS: m/z, 491.28 (M+1).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-4-(methylsulfonyl)benzohydrazide (9i)

Grey solid; Yield: 84%; M.p: 128-129°C; IR (KBr): υ_{max} 3216 (-NH str), 1651 (-C=O str) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.30 (brs, 1H), 11.10 (s, 1H), 8.48 (s, 1H), 8.34 (d, J = 5.6 Hz, 1H), 8.12 (d, J = 8.0 Hz, 3H), 8.08 (d, J = 7.6 Hz, 3H), 7.62 (d, J = 8.0 Hz, 2H), 7.54 (t, J = 8.0 Hz, 2H), 7.38 (s, 1H), 3.28 (s, 1H); LC-MS: m/z, 479.03 (M+1).

(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-4-fluorobenzohydrazide (9j) Off white solid; Yield: 86%; M.p: 100-102°C; IR (KBr): υ_{max} 3255 (-NH str), 1655 (-C=O str) cm^{-1};^{1}H NMR (400 MHz, DMSO-d_6): δ 12.12 (brs, 1H), 11.12 (s, 1H), 8.44 (s, 1H), 8.30 (d, J = 5.6 Hz, 1H), 8.00 (d, J = 7.2 Hz, 2H), 7.92 (t, J = 7.2 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.38-7.28 (m, 3H); LC-MS: m/z, 419.05(M+1).

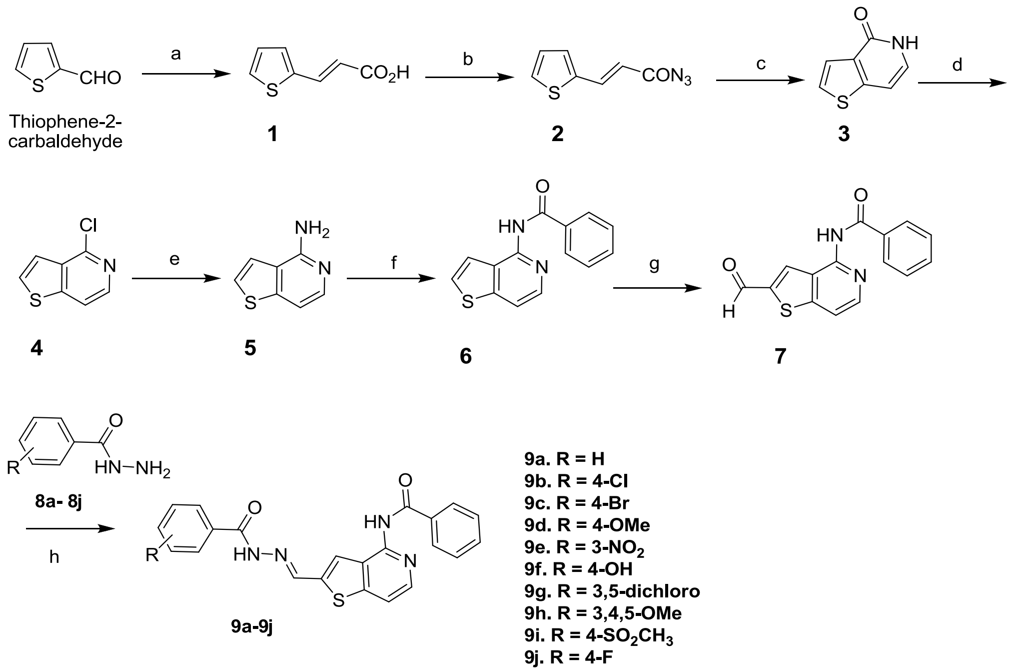
2.2 BIOLOGICAL ASSAY

The thieno[3, 2-c] pyridine hydrazide-hydrazone derivatives **9a-j,** were dissolved in dimethylsulphoxide at 50 µg/mL concentration (standard antibacterial drug, Norfloxacin was used as the reference antibiotic)and tested againstGram negative strains of*i) Escherichia coli* (MTCC 443), *(ii) Pseudomonas aeruginosa* (MTCC 424) and Gram positive strains of (iii) *Staphylococcus aureus* (MTCC 96) and *iv) Streptococcus pyogenes* (MTCC 442) using agar diffusion method according to the literature protocol.[17-18] Activity was determined by zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control. All the samples were taken in triplicates.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The preparation of thieno[3,2-c] pyridine hydrazide-hydrazone derivatives **9a-9j** is depicted in **Scheme 1**. The synthetic sequence for the preparation of hydrazide-hydrazone derivatives **9a-9j** begins with the utilization of commercially available 2-thiophenaldehyde as starting material.



Scheme 1: ***Experimental conditions:*** a) Malonic acid, TBAB, water, microwave, 100°C, 5 min; b) Cyanuric chloride, N-methylmorpholine, NaN_3, 0-5^oC, 3h; c) Tributyl amine, diphenyl ether, 230°C, 30 min; (d) Trichloroisocyanuric acid, triphenylphosphine, toluene, reflux, 5.5 h; (e) aqueous; 28% ammonia, 1,4-dioxane, 150^oC, 17 h; (f) Benzoic acid, HOBT, HBTU, DMF, rt,16 h (g) DMF, LDA, THF, - 78^oC, 10 min (h) Benzohydrazides 8a-8j, ethanol, reflux, 30 min.

Condensation of aldehyde **7** with various hydrazides **8a-j** in equimolar ratio in ethanol resulted in the formation of desired hydrazide-hydrazone derivatives **9a-9j** in quantitative yields. Knoevenagel condensation between Thiophene aldehyde and malonic acid in the presence of tetra butyl ammonium bromide (TBAB) and K_2CO_3 under microwave irradiation for 5 min at 100^{o}C in presence of water resulted in the formation of acrylic acid intermediate **1,** shorter reaction time and water as the solvent medium makes this reaction a greener protocol. Conversion of acrylic acid intermediate **1** to acyl azide **2** was accomplished under mild reaction conditions such as using NaN_3 in presence of cyanuric chloride, N-methylmorpholine in dichloromethane at room temperature for 3h. It is important to note that cyanuric chloride is a safe and inexpensive reagent in comparison to the reported use of hazardous and expensive triphosgene.[19] Curtius rearrangement of azide **2** to compounds **3** was facilitated by heating respective azide at 230^{o}C in presence of diphenyl ether. Transformation of compounds **3** to chloride intermediate **4** was accomplished using trichloroisocyanuric acid in the presence of triphenyl phosphine in refluxing toluene. The chlorination of the hydroxyheteroaromatics is usually done using $POCl_3$, $POCl_3/PCl_5$, $POCl_3/R_3N$, or NCS/PPh_3. One main drawback of using $POCl_3$ is the aqueous workup where the chloro compound can go back to the starting hydroxyhetero aromatic compound because of the heat generated in the quenching of $POCl_3$.[20] The synthesis of thieno[3,2-c]pyridine-4-amine **5** was achieved by heating chloride **4** in a steel reactor with 28% aqueous ammonia at 150^{o}C for 17 h. Amine **5** was coupled with benzoic acid in presence of HOBT, HBTU and triethyl amine in DMF to obtain amide **6**. Treatment of amide **6** with dimethyl formamide in presence of LDA in THF resulted in the formation of the key intermediate aldehyde **7**. The hydrazide derivatives 8a-8j was prepared according to the reported literature procedure.[21-23]

The structures of the synthesized compounds were confirmed by ^{1}H NMR, Mass and IR spectral data. As a representative example, the spectral analysis of **(*E*)-N'-((4-(benzamido)thieno[3,2-c]pyridin-2-yl)methylene)-3,4,5-trimethoxybenzohydrazide 9h** is described as follows: The protons resonating at 11.79 ppm (broadsinglet, 1H), 11.04 ppm (broadsinglet, 1H) and 8.81 ppm (singlet, 1H) corresponds to the groups –CO-**NH-**N=C-, -**NH**-CO-Ph and -CO-NH-N=**CH-** respectively. The protons resonating at 8.35 ppm, 7.98 ppm and 7.71 ppm as doublet, doublet and singlet corresponds to the pyridine and thiophene ring. The 3,4,5-trimethoxy phenyl ring protons resonated at 7.22 ppmas singlet with two proton integration and the benzamide ring protons appeared at 8.10 ppm (doublet, 2H), 7.64 ppm (triplet, 2H) and 7.56 ppm (triplet, 1H). Similarly, the ^{1}H NMR data of the remaining hydrazone derivatives in the series are in agreement with the assigned structures. The mass spectra of compounds showed [M+H] peaks, in agreement with their molecular formula.

3.2 Antibacterial Activity

The results of the screening of anti-bacterial data is presented in **Table 1,** it is observed that among all the hydrazide-hydrazone derivatives **9a-9j**, compound **9i** (R = 4-SO_2Me) and **9f** (R = 4-OH) exhibited excellent activity, while compounds **9d** (R = 4-OMe), **9e** (R = 3-NO_2), **9h** (R = 3,4,5-OMe), **9j** (R = 4-F), showed good activity against all the tested bacterial strains with reference to the standard drug Norfloxacin. Compounds **9a**, **9b**, **9c** and **9g** showed nil activity against all the tested above micro organisms. Anti-bacterial activity for these compounds **(9a-9j)** were not tested at higher concentrations (>50 µg/mL).

Table 1: Antibacterial Activity of Compounds 9a-9j (Concentration Used 50 µg/mL of DMSO).

Compound No.	***Gram negative***		***Gram positive***	
	E.coli MTCC 443	***P.aeruginosa MTCC 424***	***S.aureus MTCC 96***	***S.pyogenes MTCC 442***
	Zones of Inhibition of compounds 9a –9j in mm			
9a (R=H)	**-**	**-**	**-**	**-**
9b (R=4-Cl)	**-**	**-**	**-**	**-**
9c (R=4-Br)	**-**	**-**	**-**	**-**
9d (R=4-OMe)	**20**	**16**	**16**	**15**
9e (R= 3-NO_2)	**23**	**18**	**22**	**18**
9f (R= 4-OH)	**28**	**24**	**27**	**23**
9g (R=3,5-dichloro)	**-**	**-**	**-**	**-**
9h (R= 3,4,5-OMe)	**22**	**17**	**18**	**17**
9i (R= 4-SO_2Me)	**27**	**22**	**26**	**21**
9j (R= 4-F)	**23**	**18**	**22**	**18**
Standard Drug Norfloxacin (50 µg/mL).	26	21	**25**	20

4. CONCLUSION

In summary, the present paper reports an efficient synthesis of some new thieno[3,2-c]pyridine hydrazide-hydrazone derivatives (**9a-9j**) and tested for their antibacterial activity against Gram positive and Gram negative bacterial strains with Norfloxacin as standard drug. The results of the antibacterial activity data revealed that, compound **9i** (R = 4-SO_2Me) and **9f** (R = 4-OH) exhibited excellent activity, while compounds **9d** (R = 4-OMe), **9e** (R = 3-NO_2), **9h** (R = 3,4,5-OMe), **9j** (R = 4-F), showed good activity against all the tested bacterial strains with reference to the standard drug Norfloxacin. Compounds **9a**, **9b**, **9c** and **9g** showed nil activity against all the tested above micro organisms.

5. ACKNOWLEDGEMENT

One of the author (NSLR) is thankful to **Prof. Mandava V. Basaveswara Rao,** Department of Chemistry, KrishnaUniversity, Machilipatnam, A.P, India, for helpful suggestions and constant encouragement.

6. CONFLITCT OF INTEREST

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

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