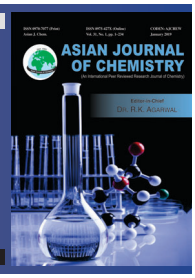
Asian Journal of Chemistry; Vol. 31, No. 3 (2019), 627-632

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.21461



Synthesis and Antibacterial Evaluation of Hydrazone Derivatives Bearing 6-Chlorothieno[3,2-*c*]pyridine Moiety

N. SREE LAKSHMANA RAO[1], MANDAVA V. BASAVESWARA RAO[2,*] and K.R.S. PRASAD[1]

[1]Department of Chemistry, Koneru Lakshmiah Education Foundation, Green Fields, Vaddeswaram-522502, India
[2]Department of Chemistry, Krishna University, Machilipatnam-521001, India

*Corresponding author: E-mail: mandavabasaveswararaov@gmail.com

Received: 19 May 2018; *Accepted*: 21 November 2018; *Published online*: 31 January 2019; AJC-19258

The present investigation describes the overall preparation of 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide-hydrazone subsidiaries (**7a-r**) from industrially accessible ethyl-4,6-dichloronicotinate as starting material. The synthesis of these derivatives involve some prominent reactions such as (i) $NaBH_4$ reduction of ethyl ester group (ii) MnO_2 oxidation of 1º alcohol (iii) cyclization of aldehyde with ethylmercapto acetate leading to thieno[3,2-*c*]pyridine ring (iv) hydrazinolysis of the ethyl group (v) condensation of selected aldehydes with 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide leading to the desired 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide-hydrazone derivatives (**7a-r**). The structure of the derivatives was characterized by elemental analysis, NMR, infrared and mass spectroscopic analysis. These compounds were screened for their antibacterial property towards Gram positive and Gram negative bacterial strains using agar diffusion method with reference to norfloxacin as reference antibiotic.

Keywords: Ethyl-4,6-dichloronicotinate, Carbohydrazide-hydrazone, Antibacterial activity, Thieno[3,2-*c*]pyridine.

INTRODUCTION

The drugs especially antibiotics related to microorganism infections presently characterize a considerable therapeutic task throughout the world. Although these antibiotics are utilized for many infectious, the influx of newest infectious diseases as well as additive levels of resistance in some pathogens-impend to weaken the success of the few remaining medication offered for the treatment of those infections [1,2]. The scarcity of innovation within the development of latest antibiotics has considerably inflated the challenge of treating and eliminating bound infecting pathogens, that cause a hour fall within the numbers of latest approvals and few novel molecules [2]. Whereas this trend is dynamic, there's an pressing demand for the event of latest medicinal drug agents with divergent and distinctive structural options that operate *via* a distinct mechanisms of action to those of existing antimicrobial agents [2,3].

In recent years, interest in one of the important hetrocyclic ring nuclei such as thieno[3,2-*c*]pyridines has emerged due to their similarity to benzofuran, quinoline and isoquinoline, which are important nuclei present in many biologically active compounds.Thiophenes with fused six membered hetrocyclic ring are currently attracting special interest. In numerous therapeutic areas, thieno[3,2-*c*]pyridine derivatives was found to exhibit various medicinal properties *viz*., antithrombin activity [4], antipsychotic activity [5] and as a protease kinase inhibitor [6]. Furthermore, in recent years, some of thieno[3,2-*c*] derivatives exhibited fluorescence properties [7,8].

Hydrazone derivatives have created a lot of widespread research in their utility and have been utilized as important precursors in the construction of various heterocyclic ring reactions [9]. Hydrazones constitute a class of organic compounds, which attracts the attention of medicinal chemists due to the fact that they contain azomethine group (-NH-N=CH-) connected with carbonyl group, which is responsible for their various biological activities [9], *viz*., anticancer [10], anti-inflammatory [11], antiviral [12]. In recent years, compounds with hydrazide-hydrazone structure have been found to exhibit antibacterial activity to a significant level [13]. Certain complexes of hydrazones catalyze physiological processes so as to get transformed to go about as sterilants for houseflies, nematocides, herbicides, bug sprays, rodenticides and plant development controllers

[14-16]. Furthermore, hydrazones discover applications in colorimetric or fluorimetric determinations, specifically, an expanding number of N-N bond-containing heterocycles and peptidomimetics have advanced into commercial applications as pharmaceutical and horticultural agents [17-19].

Inspired with the above biological activities of hydrazide-hydrazones and in continuation to previous research on thiophene-[3,2-*c*]pyridine and furo[3,2-*c*]pyridine hydrazide-hydrazone derivative [20,21], we report here in the preparation, structural determination and screening results of antibacterial activity of a new series of 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide-hydrazone derivatives (**7a-r**).

EXPERIMENTAL

Reagents and solvents obtained from commercial suppliers were used without purification or drying unless otherwise noted. ^{1}H NMR were recorded using Bruker & Agilent instrument at 400 MHz and TMS was used as an internal standard. LCMS analysis was performed on Waters UPLC with SQD-2 mass detector (single quadrupole). δ parts per million (ppm) a unit of measurement for chemical shifts are reported 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 spectra were recorded on a Perkin Elmer FT-IR spectrometer. TLC analysis was monitored used silica gel 60 F_{254} pre-coated plates prior to the use with appropriated eluents necessary for the experiments. Silica gel 60-120/100-200 mesh was used for column chromatography. Mel-temp apparatus was used for the determination of melting point and are uncorrected.

(4,6-Dichloropyridin-3-yl)methanol (2): In a mixture of tetrahydrofuran:methanol (8:2) (200 mL) was added ethyl-4, 6-dichloronicotinate (**1**) (10.0 g, 45.45 mmol) followed by $NaBH_4$ (3.45 g, 90.90 mmol) at 0 ºC. After 4 h of stirring at room temperature, the reaction mixture was quenched with water at 0 ºC and extracted with ethyl acetate. After usual work up procedure, compound **2** was isolated. Yellow solid; Yield: 7.2 g, 82 %. Elemental analysis of $C_6H_5NOCl_2$ calcd. (found) %: C 40.48 (40.50); H 2.83 (2.80); N 7.87 (7.86).

4,6-Dichloronicotinaldehyde (3): MnO_2 (21.23 g, 244 mmol) was added to a pre-mixed solution of compound **2** (7.2 g, 40.67 mmol) in methanol (140 mL). The reaction content was stirred and heated to 60 ºC for 18 h and filtered while hot and the inorganic discharge was washed with methanol. The combined filtrate was concentrated to obtain compound **3**. Yellow solid; Yield: 5 g, 70 %. Elemental analysis of $C_6H_3NOCl_2$ calcd. (found) %: C 40.95 (40.97); H 1.72 (1.70); N 7.96 (7.94).

Ethyl 6-chlorothieno[3,2-*c*]pyridine-2-carboxylate (4): A pre-mixed stirred solution of compound **3** (7.4 g, 42.28 mmol) in dichloromethane (40 mL) containing triethyl amine (11.37 mL, 84.56 mmol) was added ethylmercapto acetate (5.59 mL, 50.74 mmol) at 0 ºC. The reaction mixture was stirred at room temperature for 1 h and then to 50 ºC for 18 h. The reaction mixture was quenched with water and extracted with ethyl acetate. After usual standard work up procedure and purification by column chromatography (silica gel: 100-200 mesh, eluent: 5% ethyl acetate in pet ether) isolated compound **4**; Light Yellow solid; Yield: 5.7 g, 56 %; m.p.: 108-112 ºC. Elemental analysis of $C_{10}H_8NO_2SCl$ calcd. (found) %: C 49.69 (49.71), H 3.34 (3.32), N 5.80 (5.77)

6-Chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (5): Hydrazine hydrate monohydrate (4.88 mL, 99.58 mmol) was added to a pre-mixed solution of compound **4** (3.0 g, 12.44 mmol) in ethanol (240 mL). The reaction content was stirred at room temperature for 1 h and later heated to 80 ºC for 18 h. The reaction mixture was evaporated under reduced pressure and the residue was triturated with *n*-pentane followed by methanol to result in compound **5**. Off white solid; Yield: 2.5 g, 88 %); m.p.: 282-286 ºC; IR (KBr, ν_{max}, cm^{-1}): 3316 (-NH_2-), 1647 (-C=O), 1574 (-C=N *str.*), 1367 (C-N *str.*). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.30 (brs, 1H), 9.10 (s, 1H), 8.30 (s, 1H), 8.18 (s, 1H), 4.75 (brs, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.40, 149.58, 147.02, 145.29, 141.19, 135.32, 121.89, 117.48; ESI MS: *m/z*, 28.08 $(M+H)^+$. Elemental analysis of $C_8H_6N_3OSCl$ calcd. (found) %: C 42.20 (42.18); H 2.66 (2.68); N 18.46 (18.44).

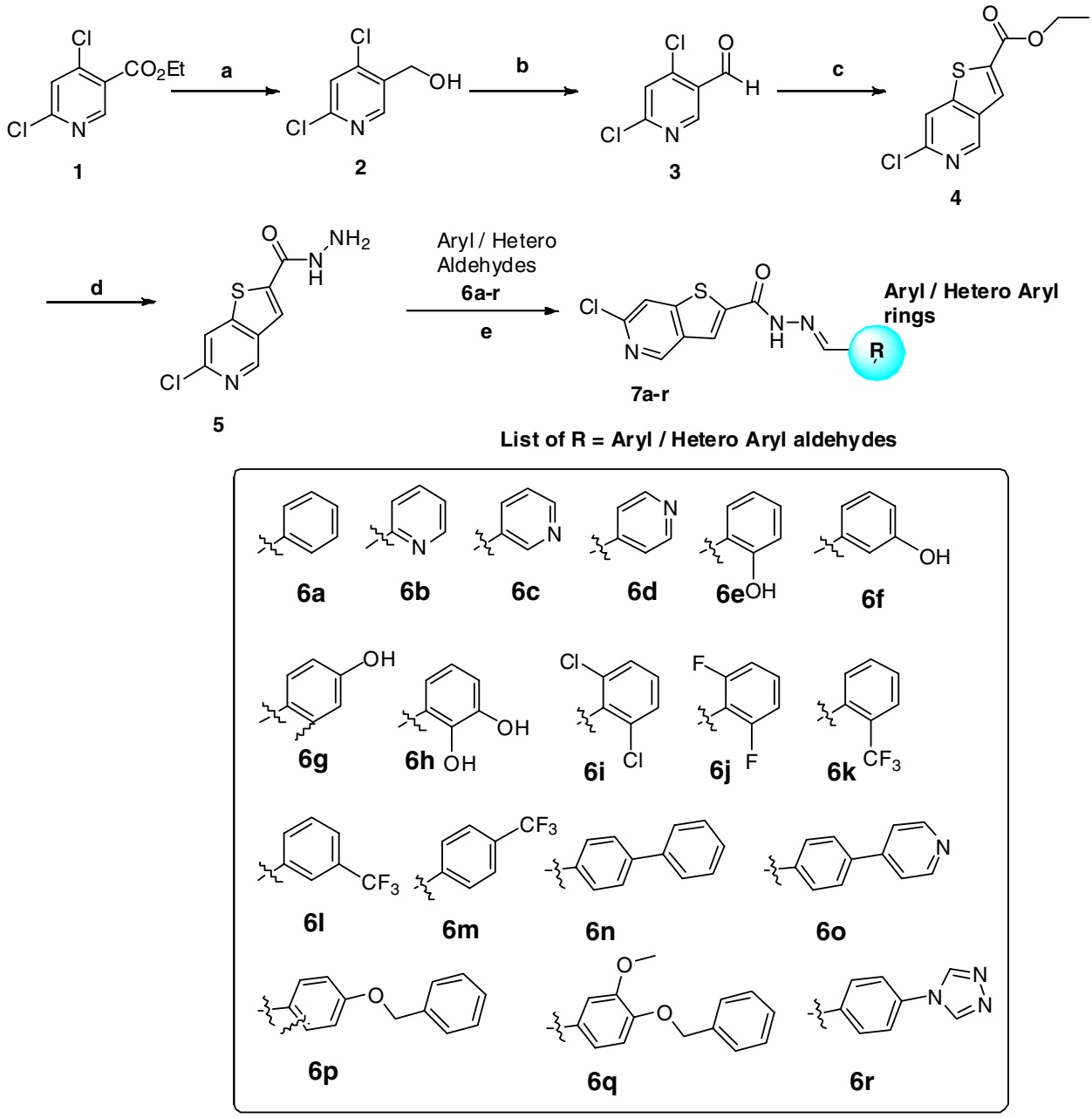
Synthesis of 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide-hydrazone derivatives (7a-r): Respective aromatic/hetro aromatic aldehydes (**6a-r**) (0.43 mmol) was added to a pre-mixed solution of compound **5** (0.43 mmol) in ethanol. The reaction content was refluxed for 0.5 h. The solids was filtered and rinsed with ethanol and dried to afford the respective hydrazone derivatives (**7a-r**) in 82-93 % yield (**Scheme-I**).

(*E*)-*N*′-Benzylidene-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7a): White solid; Yield: 120 mg, 86.5%; m.p.: 275-279 ºC; IR (KBr, ν_{max}, cm^{-1}): 3228 (-CO-NH *str.*), 1638 (-C=O *str.*), 1558 (-C=N *str.*), 1364 (-C=C *str.*), 1263 (-C-N *str.*), 636 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.32 (*12.23, s, 1H), 9.16 (d, *J* = 9.5 Hz, 1H), 8.55 (*8.48, s, 1H), 8.36 (d, *J* = 8.0 Hz, 2H), 8.19 (s, 1H), 7.87 (d, J = 7.0 Hz, 1H), 7.77 (d, *J* = 5.5 Hz, 1H), 7.54-7.48 (m, 3H); ESI MS: *m/z* 313.8 $(M-H)^+$. Elemental analysis of $C_{15}H_{10}N_3OSCl$ calcd. (found) %: C 57.05 (57.07); H 3.19 (3.21); N 13.31 (13.33).

(*E*)-6-Chloro-*N*′-((pyridin-2-yl)methylene)thieno[3,2-*c*]-pyridine-2-carbohydrazide (7b): Off white solid; Yield: 120 mg, 86.2 %; m.p. 295-298 ºC; IR (KBr, ν_{max}, cm^{-1}): 3081 (-CO-NH *str.*), 1667 (-C=O), 1554 (-C=N *str.*), 1376 (C=C *str.*), 1260 (-C-N *str.*), 638 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.47 (*12.40, s, 1H), 9.17 (s, 1H), 8.65 (d, *J* = 4.5 Hz, 1H), 8.57 (*8.49, s, 1H), 8.35 (s, 1H), 8.23 (s, 1H), 7.98-7.94 (m, 2H), 7.47 (brs, 1H); ESI MS: *m/z*, 314.3 $(M-H)^+$. Elemental analysis of $C_{14}H_9N_4OSCl$ calcd. (found) %: C 53.08 (53.05); H 2.86 (2.85); N 17.69 (17.66).

(*E*)-6-Chloro-*N*′-((pyridin-3-yl)methylene)thieno[3,2-*c*]-pyridine-2-carbohydrazide (7c): Light yellow solid; Yield: 115 mg, 82.6%; m.p.: 310-313 ºC; IR (KBr, ν_{max}, cm^{-1}): 3078 (-CO-NH *str.*),1665 (-C=O), 1552 (-C=N *str.*), 1374 (C=C *str.*), 1260 (-C-N *str.*), 636 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.46 (* 12.37, s, 1H), 9.17 (s, 1H), 9.01 (*8.90, s, 1H), 8.66 (s, 1H), 8.54 (d, *J* = 10.0 Hz, 1H), 8.37 (d, *J* = 6.0 Hz, 1H), 8.38-8.17 (m, 2H), 7.53 (brs, 1H); ESI MS: *m/z*, 316.7 $(M+H)^+$. Elemental analysis of $C_{14}H_9N_4OSCl$ calcd. (found)%: C 53.08 (53.04); H 2.86 (2.88); N 17.69 (17.66).

(*E*)-6-Chloro-*N*′-((pyridin-4-yl)methylene)thieno[3,2-*c*]-pyridine-2-carbohydrazide (7d): Off white solid; Yield: 119 mg, 85.5 %; m.p. 357-359 ºC; IR (KBr, ν_{max}, cm^{-1}): 3081



Reaction conditions: a) $NaBH_4$, THF: MeOH, 0 °C to room temperature, 4 h; b) MnO_2, methanol, 60 °C, 18 h; c) ethylmercapto acetate, triethylamine, dichloromethane, 50 °C for 8 h; d) hydrazine-hydrate, ethanol, 80 °C, 18 h; e) aromatic/hetro aromatic aldehydes **6a-r**, ethyl alcohol, 80 °C, 30 min

Scheme-I: 6-Chlorothieno[3,2-c]pyridine-2-carbohydrazide derivatives (**7a-r**)

(-CO-NH *str.*),1667 (-C=O), 1588 (-CH=CH-CO, α,β-unsaturated *str.*), 1376 (C=C *str.*). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.23 (*10.09, s, 1H), 9.18 (*9.03, s, 1H), 8.89 (d, *J* = 4.5 Hz, 1H), 8.76-8.66 (m, 1H), 8.37 (*8.29, s, 1H), 8.12 (s, 1H), 7.81 (d, *J* = 4.5 Hz, 2H); ESI MS: *m/z* 317.08 $(M+H)^+$. Elemental analysis of $C_{14}H_9N_4OSCl$, calcd. (found)%: C 53.08 (53.10); H 2.86 (2.89); N 17.69 (17.70).

(*E*)-*N*′-(2-Hydroxybenzylidene)-6-chlorothieno[3,2-*c*]-pyridine-2-carbohydrazide (7e): White solid; Yield: 120 mg, 82.3 %; m.p. 289-282 ºC; IR (KBr, ν_{max}, cm^{-1}): 3330 (-OH *str.*), 3218 (-CO-NH *str.*), 1666 (-C=O *str.*), 1588 (-C=N *str.*), 1433 (-C=C *str.*), 1378 (-C-N *str.*), 615 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.52 (*12.11, s, 1H), 9.70 (*9.65, s, 1H), 9.16 (s, 1H), 8.53 (s, 1H), 8.39-8.34 (m, 1H), 8.09 (s, 1H), 7.33-7.22 (m, 3H), 6.88 (t, *J* = 10.0 Hz, 1H); ESI MS: *m/z*, 317.08 $(M+H)^+$. Elemental analysis of $C_{15}H_{10}N_3O_2SCl$ calcd. (found) %: C 54.32 (54.30); H 3.04 (3.01); N 12.67 (12.66).

(*E*)-*N*′-(3-Hydroxybenzylidene)-6-chlorothieno[3,2-*c*]-pyridine-2-carbohydrazide (7f): Off white solid;Yield: 124 mg, 85.1 %; m.p. 290-293 ºC; IR (KBr, ν_{max}, cm^{-1}): 3322 (-OH *str.*), 3220 (-CO-NH *str.*), 1668 (-C=O *str.*), 1581 (-C=N *str.*), 1436 (-C=C *str.*), 1374 (-C-N *str.*), 621 (C-Cl) . ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.26 (*12.17, s, 1H), 9.71 (*9.69, s, 1H), 9.15 (d, *J* = 8.5 Hz,1H), 8.53 (*8.09,s, 1H), 8.38-8.31 (m, 2H), 7.31-7.13 (m, 3H), 6.87 (t, *J* = 10.0 Hz, 1H); ESI MS: *m/z*, 317.08 $(M+H)^+$. Elemental analysis of $C_{15}H_{10}N_3O_2SCl$ calcd. (found) %: C 54.28 (54.30); H 3.02 (3.01); N 12.64 (12.62).

(*E*)-*N*′-(4-Hydroxybenzylidene)-6-chlorothieno[3,2-*c*]-pyridine-2-carbohydrazide (7g): White solid; Yield: 119 mg, 81.6 %; m.p. 319-321 ºC; IR (KBr, ν_{max}, cm^{-1}): 3314 (-OH *str.*), 3234(-CO-NH *str.*), 1666 (-C=O *str.*), 1576 (-C=N *str.*), 1428 (-C=C *str.*), 1370 (-C-N *str.*), 628 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.28 (*12.10, s, 1H), 9.76 (*9.66, s, 1H), 9.18 (d, *J* = 8.5 Hz,1H), 8.50 (*8.10, s, 1H), 8.38-8.31 (m, 3H), 7.31-7.13 (m, 4H), 6.86 (t, *J* = 10.0 Hz, 1H); ESI MS: *m/z*, 317.08 $(M+H)^+$. Elemental analysis of $C_{15}H_{10}N_3O_2SCl$ calcd. (found) %: C 54.34 (54.29); H 3.06 (3.03); N 12.62 (12.60).

(*E*)-*N*′-(2,3-Dihydroxybenzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7h): Off white solid; Yield: 130 mg, 85.1 %; m.p. 318-321 ºC; IR (KBr, ν_{max}, cm^{-1}): 3343 (-OH *str.*), 3222(-CO-NH *str.*), 1668 (-C=O *str.*), 1572 (-C=N *str.*), 1417 (-C=C *str.*), 1366 (-C-N *str.*), 632 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.52 (*12.11, s, 1H), 10.67 (*9.71, s, 1H), 9.33 (*9.11, s, 1H), 9.16 (s, 1H), 8.65 (*8.54, s, 1H), 8.36 (d, *J* = 4.0 Hz, 2H), 7.44 (*7.05, d, *J* = 6.0 Hz, 1H), 6.89-6.74 (m, 2H); ESI MS: *m/z*, 345.7 $(M+H)^+$. Elemental analysis of $C_{15}H_{10}N_3O_3SCl$ calcd. (found) %: C 51.80 (51.83); H 2.90 (2.89); N 12.08 (12.10).

(*E*)-*N*′-(2,6-Dichlorobenzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7i): Off white solid; Yield: 140 mg, 82.8 %; m.p. 289-292 ºC; IR (KBr, ν_{max}, cm^{-1}): 3162 (-CO-NH *str.*), 1657 (-C=O *str.*), 1575 (-C=N *str.*), 1409 (-C=C *str.*), 1360 (-C-N *str.*), 717 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.56 (*12.42, s, 1H), 9.12 (br.s, 1H), 8.68 (*8.56, s, 1H), 8.38 (t, *J* = 10.5 Hz, 1H), 8.29 (s, 1H), 7.60 (s, 2H), 7.48 (s, 1H); ESI MS: *m/z*, 385.96 $(M+H)^+$. Elemental analysis of $C_{15}H_8N_3OSCl_3$ calcd. (found) %: C 46.84 (46.80), H 2.10 (2.12), N 10.92 (10.95).

(*E*)-*N*′-(2,6-Difluorobenzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7j): Light yellow solid; Yield: 137 mg, 88.6 %; m.p.: 253-256 ºC; IR (KBr, ν_{max}, cm^{-1}): 3222 (-CO-NH *str.*), 1668 (-C=O *str.*), 1572 (-C=N *str.*), 1422 (-C=C *str.*), 1368 (-C-N *str.*), 628 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.45 (*12.31, s, 1H), 9.15 (*9.10, s, 1H), 8.64 (*8.60, s, 1H), 8.37-8.24 (m, 2H), 7.56 (d, *J* = 6.4 Hz, 1H), 7.26 (t, *J* = 9.2 Hz, 2H); ESI MS: *m/z*, 352.05 $(M+H)^+$. Elemental analysis of $C_{15}H_8N_3OSClF_2$ calcd. (found) %: C 51.22 (51.20); H 2.29 (2.27); N 11.95 (11.97).

(*E*)-*N*′-(2-(Trifluoromethyl)benzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7k): Off white solid; Yield: 139 mg, 82.4 %; m.p.: 292-294 ºC; IR (KBr, ν_{max}, cm^{-1}): 3218 (-CO-NH *str.*), 1667 (-C=O *str.*), 1575 (-C=N *str.*), 1428 (-C=C *str.*), 1366 (-C-N *str.*), 626 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.58 (*12.46, s, 1H), 9.16 (d, *J* = 10.5 Hz, 1H), 8.84 (s, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 8.38-8.35 (m, 3H), 8.24 (d, *J* = 7.5 Hz, 2H); ESI MS: *m/z*, 383.60 $(M+H)^+$. Elemental analysis of $C_{16}H_9N_3OSClF_3$ calcd. (found) %: C 50.07 (50.11); H 2.36 (2.38); N 10.95 (10.97).

(*E*)-*N*′-(3-(Trifluoromethyl)benzylidene)-6-chlorothieno [3,2-*c*]pyridine-2-carbohydrazide (7l): Off white solid; Yield: 142 mg, 84.24 %; m.p.: 267-269 ºC; IR (KBr, ν_{max}, cm^{-1}): 3165 (-CO-NH *str.*), 1663 (-C=O *str.*), 1573 (-C=N *str.*), 1435 (-C=C *str.*), 1392 (-C-N *str.*), 1119 (C-F), 666 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.53 (*12.43, s, 1H), 9.16 (s, 1H), 8.55 (s, 1H), 8.40-8.25 (m, 3H), 8.12-8.07 (m, 2H), 7.84 (d, *J* = 6.4 Hz, 1H), 7.76-7.71 (m, 1H); ESI MS: *m/z*, 384.11 $(M+H)^+$. Elemental analysis of $C_{16}H_9N_3OSClF_3$ calcd. (found) %: C 51.06 (51.08); H 2.28 (2.31); N 11.88 (11.91).

(*E*)-*N*′-(4-(Trifluoromethyl)benzylidene)-6-chlorothieno-[3,2-*c*]pyridine-2-carbohydrazide (7m): Off white solid; Yield: 150 mg, 88.98 %; m.p.: 309-312 ºC; IR (KBr, ν_{max}, cm^{-1}): 3160 (-CO-NH *str.*), 1664 (-C=O *str.*), 1571 (-C=N *str.*), 1432 (-C=C *str.*), 1392 (-C-N *str.*), 1119 (C-F), 666 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.51 (*12.40, s, 1H), 9.18 (s, 1H), 8.53 (s, 1H), 8.38-8.23 (m, 3H), 8.10-8.05 (m, 2H), 7.85 (d, *J* = 6.4 Hz, 1H), 7.74-7.70 (m, 1H); ESI MS: *m/z*, 384.11 $(M+H)^+$. Elemental analysis of $C_{16}H_9N_3OSClF_3$ calcd. (found) %: C 50.27 (50.24); H 2.34 (2.30); N 10.97 (10.88).

(*E*)-*N*′-([1,1′-Biphenyl]-4-ylmethylene)-6-chlorothieno-[3,2-c]pyridine-2-carbohydrazide (7n): Light yellow solid; Yield: 142 mg, 82.7%; m.p.: 294-296 ºC; IR (KBr, ν_{max}, cm^{-1}): 3155 (-CO-NH*str.*), 1654 (-C=O *str.*), 1573 (-C=N *str.*), 1400 (-C=C *str.*), 1340 (-C-N *str.*), 572 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.36 (*12.28, s, 1H), 9.18 (*9.15, s, 1H), 8.56 (*8.52, s, 1H), 8.38 (*8.23, s, 1H), 8.36 (s, 1H), 7.96 (d, *J* = 10.5 Hz, 1H), 7.87-7.79 (m, 3H), 7.75 (d, *J* = 9.5 Hz, 2H), 7.51 (t, *J* = 9.0 Hz, 2H), 7.43 (d, *J* = 8.5 Hz, 1H); ESI MS: *m/z*, 392.10 $(M+H)^+$. Elemental analysis of $C_{21}H_{14}N_3OSCl$ calcd. (found) %: C 64.36 (64.33); H 3.60 (3.59); N 10.72 (10.74).

(*E*)-*N*′-(4-(Pyridin-4-yl)benzylidene)-6-chlorothieno-[3,2-*c*]pyridine-2-carbohydrazide (7o): Off white solid; Yield: 160 mg, 93 %; m.p.: 325-328 ºC; IR (KBr, ν_{max}, cm^{-1}): 3155 (-CO-NH *str.*), 1661 (-C=O *str.*), 1585 (-C=N *str.*), 1506 (-C=C *str.*), 1398 (-C-N *str.*), 671 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.42 (*12.33, s, 1H), 9.17 (d, *J* = 7.4 Hz, 1H), 8.68 (s, 2H), 8.57 (*8.25, s, 2H), 8.54 (*8.39, s, 2H), 8.03-7.91 (m, 3H), 7.78 (d, *J* = 6.0 Hz, 2H); ESI MS: *m/z*, 392.17 $(M+H)^+$. Elemental analysis of $C_{20}H_{13}N_4OSCl$ calcd. (found) %: C 61.14 (61.11); H 3.34 (3.37); N 14.26 (14.28).

(*E*)-*N*′-(4-(Benzyloxy)benzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7p): Light yellow solid; Yield: 171 mg, 92.4 %; m.p.: 262-266 ºC; IR (KBr, ν_{max}, cm^{-1}): 3162 (-CO-NH *str.*), 1664 (-C=O), 1575 (-C=N *str.*), 1506 (-C=C *str.*), 1389 (C-NC *str.*), 676 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.20 (*12.11, s, 1H), 9.15 (d, *J* = 8.0 Hz, 1H), 8.53 (*8.41, s, 1H), 8.34 (s, 1H), 8.12 (s, 1H), 7.81 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.48-7.35 (m, 5H), 7.14 (t, *J* = 6.4 Hz, 2H); ESI MS: *m/z*, 412.7 $(M+H)^+$. Elemental analysis of $C_{22}H_{16}N_3O_2SCl$, calcd. (found)%: C 62.63 (62.65); H 3.82 (3.85); N 9.96 (9.97).

(*E*)-*N*′-(4-(Benzyloxy)-3-methoxybenzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7q): Off white solid; Yield: 169 mg, 85.3 %; m.p.: 212-216 ºC; IR (KBr, ν_{max}, cm^{-1}): 3154 (-CO-NH *str.*), 1652 (-C=O *str.*), 1577 (-C=N *str.*), 1508 (-C=C *str.*), 1391 (-C-N *str.*), 1271 (C-O-C-l), 615 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.21 (*12.18, s, 1H), 9.14 (s, 1H), 8.54 (*8.40, s, 1H), 8.34 (d, *J* = 9.0 Hz, 1H), 8.09 (s, 1H), 7.52 (d, *J* = 1.5 Hz, 1H), 7.48 (d, *J* = 2.0 Hz, 2H), 7.46-7.35 (m, 5H), 7.14 (d, *J* = 10.5 Hz, 1H), 5.17 (*5.16, s, 2H), 3.92 (*3.84, s, 3H); ESI MS: *m/z*, 452.15 $(M+H)^+$. Elemental analysis of $C_{23}H_{18}N_3O_3SCl$ calcd. (found)%: C 61.13 (61.10); H 4.01 (3.99); N 9.30 (9.28).

(*E*)-*N*′-(4-(4*H*-1,2,4-Triazol-4-yl)benzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (7r): Off white solid; Yield: 152 mg, 90.58 %; m.p.: 350-353 °C; IR (KBr, ν_{max}, cm^{-1}):

3155 (-CO-NH *str.*), 1661 (-C=O *str.*), 1585 (-C=N *str.*), 1506 (-C=C *str.*), 1398 (-C-N *str.*), 671 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.42 (*12.34, s, 1H), 9.48 (s, 1H), 9.42 (s, 1H), 8.57-8.52 (m, 1H), 8.39-8.30 (m, 3H), 8.07-7.95 (m, 4H); ESI MS: *m/z*, 383.12 $(M+H)^+$. Elemental analysis of $C_{17}H_{11}N_6OSCl$ calcd. (found) %: C 53.34 (53.37); H 2.90 (2.87); N 21.95 (21.92).

Biological assay: The antibacterial activity experiments were carried out following our previously reported procedure [20,21]. The 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide-hydrazone derivatives (**7a-r**) (50 µg/mL concentration) were tested in contradiction to *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442) belonging to Gram negative strain and *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) belonging to Gram positive strain, utilizing a standard methodology *viz*., agar diffusion method where in norfloxacin was used as the reference antibiotic.

RESULTS AND DISCUSSION

Sodium borohydride reduction of ethyl-4,6-dichloronicotinate (1) in THF:methanol at 0 ºC to room temperature for 4 h gave (4,6-dichloropyridin-3-yl)methanol (**2**). MnO_2 oxidation of alcohol **2** in methanol at 60 ºC for 18 h produced 4,6-dichloronicotinaldehyde (**3**). Condensation of aldehyde **3** with ethyl mercapto acetate in presence of dichloromethane at 50 ºC for 8 h gave cyclized ethyl ester intermediate **4**. Hydrazinolysis of ethyl ester **4** in ethanol at 80 ºC for 18 h gave the desired product carbohydrazide [5]. Condensation of carbohydrazide **5** with various aromatic/hetro aromatic aldehydes **6a-r** at reflux in ethanol for 30 min produced 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide-hydrazone derivatives (**7a-r**).

The synthesized hydrazone derivatives of (*E*)-*N*′-(2,6-dichlorobenzylidene)-6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide (**7a-r**) were sufficiently characterized using ^{1}H NMR, mass and IR spectroscopic techniques. These hydrazone derivatives were found to exist as a mixture of two rotameric forms in solution [22,23] as indicated by their ^{1}H NMR spectrum. The basis of the '*E*' geometry of C=N bond by ^{1}H NMR was established based on the reported literature data [24,25].

As a representative example, structural interpretation of (*E*)-*N*′-(2,6-dichlorobenzylidene)-6-chlorothieno[3,2-*c*]-pyridine-2-carbohydrazide **(7i)**, is described here, the protons resonating at 9.12 ppm appeared as singlet (1H integration) and the proton resonating at 8.29 ppm as singlet (1H integration) corresponds to the pyridine ring and proton signal at 7.48 ppm as singlet with (1H integration) is assigned to the fused thiophene ring while the protons signals resonating at 12.56 (*12.42, singlet, 1H) ppm and 8.68 (*8.56, singlet, 1H) ppm corresponds to -CO-N<u>H</u>- and -N=C<u>H</u>- groups in the scaffold, respectively. Finally, the proton signals resonating at 8.38 ppm (triplet, 1H) and 7.60 ppm (singlet, 2H) corresponds to 2,6-chlorophenyl ring system. The mass spectrum of compound **7i** showed a molecular ion peak at *m/z* 385.96 and is in agreement with the desired molecular formula. The distinguished absorption peaks in the region 3162, 1657, 1575, 1360 cm^{-1} confirms the presence of the desired functional group -CONH-, -C=O, -C=N and -C-N, respectively as indicated from the IR spectra of compound **7i**. Based on the above analytical description, the structure of compound **7i** was determined. Similarly, the remaining compounds **7a-r** have been fully characterized as per the above description.

Antibacterial activity: The zone of inhibition values of 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide-hydrazone derivatives (**7a-r**) obtained from the antibacterial experimentation is given in Table-1. It is evident that compound **7b** (R = 2-substituted pyridine), **7c** (R = 3-substituted pyridine), **7d** (R = 4-substituted pyridine)**, 7o** (R = 4-phenyl pyridine), **7r** (R = 4-phenyl-4*H*-1,2,4-triazole) exhibited good antibacterial activity. Compounds **7e** (R = *o*-hydroxyphenyl), **7f** (R = *m*-hydroxyphenyl), **7g** (R = *p*-hydroxyphenyl), **7h** (R = *o*, *m*-hydroxyphenyl), **7k** (R = *o*-CF_3 Phenyl), **7l** (R = *m*-CF_3 phenyl) and **7m** (R = *p*-CF_3 phenyl) moderate antibacterial activity, while compounds **7a** (R = phenyl), **7i** (R = 2,6-dichloro phenyl), **7j** (R = 2,6-difluorophenyl) showed weak antibacterial activity. Finally, compounds **7n** (R = biphenyl), **7p** (R = (phenoxy-methyl)-benzene) and **7q** (R = (2-methoxyphenoxy)methyl) benzene) did not show any antibacterial activity as they exhibited zero zone of inhibition.

TABLE-1
ZONE OF INHIBITION VALUES OF COMPOUNDS **7a-r** (50 µg/mL) FOR THE DETERMINATION OF ANTIBACTERIAL ACTIVITY

Compd. No.	Gram-negative		Gram-positive	
	E. coli	*P. aeruginosa*	*S. aureus*	*S. pyogenes*
7a	8	7	7	6
7b	24	19	22	18
7c	22	17	21	17
7d	21	18	20	17
7e	15	11	14	10
7f	16	14	16	13
7g	16	13	16	12
7h	15	14	15	13
7i	11	10	11	8
7j	10	7	6	5
7k	16	15	12	14
7l	15	14	14	12
7m	16	13	15	11
7n	–	–	–	–
7o	22	18	20	18
7p	–	–	–	–
7q	–	–	–	–
7r	25	19	23	18
*Standard drug	26	21	25	20

Conclusion

The synthesis, characterization and antibacterial activity of some new hydrazone derivatives (**7a-r**) obtained from condensation of 6-chlorothieno[3,2-*c*]pyridine-2-carbohydrazide and corresponding aromatic and hetroaromatic aldehydes. The antibacterial results of the study demonstrated that compound **7b** (R = 2-substituted pyridine), **7c** (R = 3-substituted pyridine), **7d** (R = 4-substituted pyridine), **7o** (R = 4-phenyl pyridine), **7r** (R = 4-phenyl-4*H*-1,2,4-triazole) exhibited good antibacterial activity. Compounds **7e** (R = *o*-hydroxyphenyl), **7f** (R = *m*-hydroxyphenyl), **7g** (R = *p*-hydroxyphenyl), **7h** (R = *o*, *m*-hydroxyphenyl), **7k** (R = -*o*-CF_3 Phenyl), **7l** (R = *m*-CF_3 phenyl) and **7m** (R = *p*-CF_3 phenyl) moderate antibacterial activity, while the compounds **7a** (R = phenyl), **7i** (R = 2,6-dichloro-

phenyl), **7j** (R = 2,6-difluorophenyl) showed weak antibacterial activity. Finally, compounds **7n** (R = biphenyl), **7p** (R = (phenoxymethyl)benzene) and **7q** (R = (2-methoxyphenoxy)-methyl)benzene) did not show any antibacterial activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. I. Chopra, C. Schofield, M. Everett, A. O'Neill and K. Miller, *Lancet Infect. Dis*., **8**, 133 (2008); https://doi.org/10.1016/s1473-3099(08)70018-5.
2. K.M. Overbye and J.F. Barrett, *Drug Discov. Today*, **10**, 45 (2005); https://doi.org/10.1016/s1359-6446(04)03285-4.
3. C.B. Sangani, D.C. Mungra, M.P. Patel and R.G. Patel, *Chin. Chem. Lett.*, **23**, 57 (2012); https://doi.org/10.2478/s11532-011-0041-7.
4. J.S. New, W.L. Christopher, J.P. Yevich, R. Butter, R.S. Schlemmer Jr., C.P. Van der Maelen and J.A. Cipolline, *J. Med. Chem*., **32**, 1147 (1989); https://doi.org/10.1021/jm00126a002.
5. Y. Miyazaki, M. Nakano, H. Sato, A.T. Truesdale, J.D. Stuart, E.N. Nartey, K.E. Hightower and L. Kane-Carson, *Bioorg. Med. Chem. Lett.*, **17**, 250 (2007); https://doi.org/10.1002/chin.200719144.
6. A. Khalafi-Nezhad, M.N.S. Rad, H. Mohabatkar, Z. Asrari and B. Hemmateenejad, *Bioorg. Med. Chem.*, **13**, 1931 (2005); https://doi.org/10.1016/j.bmc.2005.01.014.
7. J.B.M. Rewinkel, H. Lucas, M.J. Smit, A.B.J. Noach, T.G. van Dinther, A.M.M. Rood, A.J.S.M. Jenneboer and C.A.A. van Boeckel, *Bioorg. Med. Chem. Letts.*, **9**, 2837 (1999); https://doi.org/10.1016/s0960-894x(99)00483-7.
8. R.B. Toche and S.N. Chavan, *J. Fluoresc*., **24**, 285 (2014); https://doi.org/10.1007/s10895-013-1313-8.
9. S. Rollas and S.G. Küçükgüzel, *Molecules*, **12**, 1910 (2007); https://doi.org/10.3390/12081910.
10. T. Nasr, S. Bondock and M. Youns, *Eur. J. Med. Chem*., **76**, 539 (2014); https://doi.org/10.1016/j.ejmech.2014.02.026.
11. V. Kumar, G. Basavarajaswamy, M.V. Rai, B. Poojary, V.R. Pai, N. Shruthi and M. Bhat, *Bioorg. Med. Chem. Lett.*, **25**, 1420 (2015); https://doi.org/10.1016/j.bmcl.2015.02.043.
12. S. Senkardes, N. Kaushik-Basu, I. Durmaz, D. Manvar, A. Basu, R. Atalay and S.G. Kücükgüzel, *Eur. J. Med. Chem*., **10**, 301 (2016); https://doi.org/10.1016/j.ejmech.2015.10.041.
13. L. Popiolek. *Med. Chem. Res*., **26**, 287 (2017); https://doi.org/10.1007/s00044-016-1756-y.
14. M. Katyal and Y. Dutt, *Talanta*, **22**, 151 (1975); https://doi.org/10.1016/0039-9140(75)80161-5.
15. M. Mohan, M.P. Gupta, L. Chandra and N.K. Jha, *Inorg. Chim. Acta*, **151**, 61 (1988); https://doi.org/10.1016/S0020-1693(00)83485-4.
16. R.B. Singh, P. Jain and R.P. Singh, *Talanta*, **29**, 77 (1982); https://doi.org/10.1016/0039-9140(82)80024-6.
17. Zh.V. Molodykh, B.I. Buzykin and M.P. Kolov, *Zh. Obshch. Khim.*, **60**, 222 (1990).
18. P. Vicini, F. Zani, P. Cozzini and I. Doytchinova, *Eur. J. Med. Chem*., **37**, 553 (2002); https://doi.org/10.1016/s0223-5234(02)01378-8.
19. M.G. Mamolo, V. Falagiani, D. Zampieri, L. Vio and E. Banfi, *IL Farmaco*, **56**, 587 (2001); https://doi.org/10.1016/s0014-827x(01)01097-7.
20. N.S. Lakshmana Rao, V.M. Basaveswara Rao and K.R.S. Prasad, *World J. Pharm. Res.*, **6**, 914 (2017).
21. N.S. Lakshmana Rao and V.M. Basaveswara Rao, *Heterocycl. Lett.*, **5**, 543 (2015).
22. P. Sarbani, *J. Chem. Pharm. Res.*, **2**, 387 (2010); https://doi.org/10.1002/ardp.18601510358.
23. R.B. Palmer and N.H. Andersen, *Bioorg. Med. Chem. Lett*., **6**, 2173 (1996); https://doi.org/10.1016/0960-894X(96)00396-4.
24. R. Ivan, E. Felismino, L. Sang, N. Ken-ichi and K. Isao, *Bioorg. Med. Chem*., **15**, 6236 (2007); https://doi.org/10.1016/j.bmc.2007.06.022.
25. G. Palla, G. Predieri and P. Domiano, *Tetrahedron*, **42**, 3649 (1986); https://doi.org/10.1016/s0040-4020(01)87332-4.