Bulletin of Faculty of Pharmacy, Cairo University (2016) **54**, 77–86

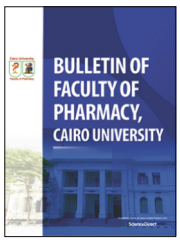
Cairo University

Faculty of Pharmacy

Cairo University

Bulletin of Faculty of Pharmacy, Cairo University

www.elsevier.com/locate/bfopcu
www.sciencedirect.com



ORIGINAL ARTICLE

Facile synthesis, spectral characterization, antimicrobial and *in vitro* cytotoxicity of novel N^3, N^5-diisonicotinyl-2,6-dimethyl-4-phenyl-1, 4-dihydropyridine-3,5-dicarbohydrazide derivatives

Karthikeyan Elumalai [a,b,*], Manogaran Elumalai [c], Kalpana Eluri [c], Sivaneswari Srinivasan [d], Mohamed Ashraf Ali [a], Basu Venkateswara Reddy [b], Sarada Prasad Sarangi [b]

[a] *New Drug Discovery Research, Department of Medicinal Chemistry, Sunrise University, Alwar, Rajasthan 301030, India*
[b] *Department of Medicinal Chemistry, Santhiram College of Pharmacy, Nandyal 518 112, India*
[c] *Faculty of Pharmaceutical Sciences, UCSI University, Cheras, Kuala Lumpur 56000, Malaysia*
[d] *Department of Pharmaceutics, K.K. College of Pharmacy, Chennai 600 122, India*

Received 9 June 2015; revised 9 December 2015; accepted 20 January 2016
Available online 10 February 2016

KEYWORDS

Isoniazid;
1,4-Dihydropyridines;
B. subtilis;
E. coli;
Vero cells

Abstract A new series of some novel isoniazid condensed 1,4-dihydropyridines was prepared by reaction of N-(3-oxobutanoyl) isonicotinohydrazide with aryl aldehyde and 25–30% ammonia solution in the presence of catalytic amount of barium nitrate as an efficient catalyst. The confirmation of the chemical structure of the synthesized compounds (4a–m) was substantiated by TLC, different spectral data IR, ^{1}H NMR, ^{13}C NMR, and mass spectra and elemental analysis was done. The synthesized compounds were evaluated for antimicrobial activity and cytotoxicity against Gram-positive bacteria *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli* and *Vero cells*. All the reported compounds exhibited weak, moderate, or high antimicrobial activity and cytotoxicity. Especially, compound 4i showed the best antimicrobial activity and cytotoxicity of all the 1,4-dihydropyridine derivatives, with an MIC value of 11.5 μM, 12.2 μM and a CTC_{50} value of 27 μM.

1. Introduction

Infectious diseases caused by microorganisms are one of the main reasons of illness in the world. The search for new antibacterial drugs is a never ending story because of the increasing resistance of the microbial pathogens.[1] Despite many available antibiotics and chemotherapeutic agents

* Corresponding author at: New Drug Discovery Research, Department of Medicinal Chemistry, Sunrise University, Alwar, Rajasthan 301030, India. Mobile: +91 95733 96024.
E-mail address: karthikeyanelumalai@hotmail.com (K. Elumalai).
Peer review under responsibility of Faculty of Pharmacy, Cairo University.

http://dx.doi.org/10.1016/j.bfopcu.2016.01.003
1110-0931

available, the emergence of old and new antibiotic resistant bacterial strains in the last decades leads to a substantial need for new classes of antibacterial agents.[2] The increasing incidence of infection caused by the rapid development of bacterial resistance to most of the known antibiotics is a serious health problem now a days. While many factors may be responsible for mutations of the microbial genomes, it has been widely demonstrated that the incorrect use of antibiotics can greatly increase the development of resistant genotypes. As the multidrug-resistant bacterial strains proliferate; the necessity for effective therapy has stimulated research into the design and synthesis of novel antimicrobial molecules.[3]

The credit of the first synthesis of dihydropyridine is attributed to Arthur Hantzsch for work performed a century ago.[4] The Hantzsch 1,4-dihydropyridines (DHPs) synthesis involves the reaction of 1,3-dicarbonyl compounds with aldehyde and ammonia. During the past decades, the scope of the original cyclocondensation reaction was gradually extended by variation of all three building blocks, allowing access to a large number of structurally diverse multifunctionalized DHPs. The discovery of a dihydropyridine (dihydronicotinamide derivative, NADH), "hydrogen transferring coenzyme" at 1930s consequently became important in the biological system. Therefore, various studies have been generated on the biochemical properties of dihydropyridine and their bioisosteres dihydropyrimidines.[5] When substituent's on the left side differ from the right side of a 1,4-DHPs, the molecule is chiral, with C(4) as the stereogenic center. The enantiomers of an unsymmetrical 1,4-DHPs usually differ in their biological activities and could even have an exact opposite activity profile.[6]

The 1,4-dihydropyridine derivatives are of interest because of their potential biological activity and use in therapeutics such as antimicrobial,[7,8] antihypertensive,[9] anticonvulsant,[10] anti-inflammatory,[11] antioxidant,[12,13] anticancer,[14–17] anticoagulant,[18] antitubercular, and[19–21] anti viral agents,[22] and calcium channel modulators of the nifedipine type.[23–26] The chemical structure of isoniazid provides a most valuable molecular template for the development of agents able to interact with a wide variety of biological activities.[27–29] Hence, it was thought worthwhile to synthesize new molecules by incorporating isoniazid with 1,4-dihydropyridines moieties in a single molecular framework and to evaluate their antimicrobial activity and cytotoxicity.

2. Experimental

2.1. Materials and methods

All chemicals were supplied by E. Merck (Germany) and S.D fine chemicals (India). Melting points were determined by the open tube capillary method and are uncorrected. The purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel GF) in the solvent system, ethanol, chloroform, ethyl acetate (4:3:3); the spots were located under iodine vapors or UV light. The IR spectrums were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). The ^{1}H NMR spectra were recorded or a Bruker DRX-300 (300 MHz FT-NMR) spectrometer using DMSO-d_6 as solvent and TMS as internal standard. The ^{13}C NMR spectra was recorded on AV-III 400 MHz spectrometer using DMSO-d_6 as solvent and TMS as internal standard. The chemical shifts are expressed in δ ppm and the following abbreviations are used; *s* = singlet, *bs* = broad singlet, *d* = doublet, *t* = triplet, *m* = multiplet. Mass spectra were obtained using Shimadzu LCMS 2010A under ESI ionization technique. Elemental analyses (C, H, and N) were performed on Perkin Elmer model 240C analyzer.

2.2. Preparation of N-(3-oxobutanoyl) isonicotinohydrazide (3)

A mixture of isoniazid 1, ethyl acetoacetate (0.01 M) and a catalytic amount of potassium t-butoxide in 95% ethanol was refluxed for 3 h. The formed reddish brown liquid was then heated on a water bath to remove the alcohol formed during the reaction. After allowing the reaction mixture to cool, crude crystals were obtained. Purification was performed by stirring crude crystals with cold diethyl ether for approximately 10 min by using a mechanical stirrer. Allowing it to stand for 15 min, followed by filtration, resulted in the third compound in a pure form of N-(3-oxobutanoyl) isonicotinohydrazide **3**.

2.3. General procedure for the preparation of 1,4-dihydropyridines by one pot-multicomponent, Hantzsch method of synthesis

The mixture of N-(3-oxobutanoyl) isonicotinohydrazide **3** (0.01 M), appropriate aldehyde (0.005 M), a catalytic amount of barium nitrate and 3 ml of 25–30% aqueous ammonia solution were transferred to a round bottom flask containing 15 ml of ethanol. The reaction mixture was refluxed for 11–18 h. One milliliter (ml) of 25% aqueous ammonia solution was added for every 3 h during the reflux. The reactions were monitored through TLC using a suitable solvent system. Soon after the reaction was completed, the reaction mixture was allowed to cool. The solid product formed was filtered and washed with cold methanol to get Hantzsch 1,4-dihydropyridines.

2.4. Analytical data

2.4.1. N-(3-oxobutanoyl) isonicotinohydrazide 3

Reddish-brown solid, M.P: 171–173 °C; yield: 63%; IR (KBr, cm^{-1}): 3364 (N—H), 2981 (Ali-C—H), 1731 (C═O, ketone), 1674 (C═O, amide), 1567 (C═C), 1344 (C—N); ^{1}H NMR (DMSO-d_6) δ: 2.03 (*s*, 3H, CH_3), 3.38 (*s*, 2H, CH_2), 7.92 (*d*, 2H, Ar-H), 8.02 (*s*, 1H, NH), 8.14 (*s*, 1H, NH), 9.01 (*d*, 2H, Ar-H); MS (m/z): (M + 1) calculated 221.07; found 221.12; calculated for $C_{10}H_{11}N_3O_3$: C, 54.24; H, 5.01; N, 19.00; found C, 54.29; H, 5.07; N, 19.02.

2.4.2. N^3,N^5-diisonicotinyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (4a)

Dark-brownish solid; M.P: 257–259 °C; yield-56%; IR (KBr, cm^{-1}): 3294 (N—H), 3081 (Ar-C—H), 3010 (Ali-C—H), 1667 (C═O, amide), 1486 (Ar-C═C), 1328 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.78 (*s*, 6H, $(CH_3)_2$), 4.58 (*s*, 1H, CH), 6.21 (*bs*, 1H, NH), 7.04–7.15 (*m*, 5H, Ar-H), 7.94 (*d*, 2H, (Ar-H)), 8.01 (*d*, 2H, (Ar-H)), 8.08 (*s*, 1H, NH), 8.13 (*s*, 1H, NH), 8.17 (*s*, 1H, NH), 8.22 (*s*, 1H, NH), 9.03 (*d*, 2H, (Ar-H)), 9.10 (*d*, 2H, (Ar-H)); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.9 (CH_3), 17.0 (CH_3), 45.1 (CH), 102.2 (2ArC), 123.4 (4ArC), 125.8 (1ArC), 128.2 (2ArC), 129.1 (2ArC), 140.9

(2ArC), 142.2 (ArC), 149.5 (2ArC), 149.8 (4ArC), 164.9 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 511.19; found 511.13; calculated for $C_{27}H_{25}N_7O_4$: C, 63.40; H, 4.93; N, 19.17; found C, 63.46; H, 4.99; N, 19.23.

2.4.3. N^3,N^5-diisonicotinyl-2,6-dimethyl-4-styryl-1,4-dihydropyridine-3,5-dicarbohydrazide (4b)

Light gray-colored solid; M.P: 238–240 °C; yield-71%; IR (KBr, cm^{-1}): 3286 (N—H), 3092 (Ar-C—H), 2994 (Ali-C—H), 1664 (C═O, amide), 1468 (Ar-C═C), 1367 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.74 (*s*, 6H, $(CH_3)_2$), 3.94 (*s*, 1H, Ali-CH), 6.05 (*s*, 1H, Ali-C—H), 6.16 (*bs*, 1H, NH), 6.38 (*s*, 1H, Ali-C—H), 7.12 (*s*, 1H, Ar-H), 7.25 (*d*, 2H, Ar-H), 7.38 (*s*, 1H, Ar-H), 7.96 (*d*, 2H (Ar-H)), 8.12 (*s*, 1H, NH), 8.19 (*s*, 1H, NH), 8.27 (*s*, 1H, NH), 8.34 (*s*, 1H, NH), 9.13 (*d*, 2H, (Ar-H)); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.9 (CH_3), 17.2 (CH_3), 31.2 (CH), 102.7 (2ArC), 121.7 (═C), 122.8 (4ArC), 126.4 (2ArC), 128.1 (ArC), 128.6 (2ArC), 130.5 (═C), 135.2 (ArC), 139.8 (2ArC), 149.5 (2ArC), 149.9 (4ArC), 164.9 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 537.21; found 537.24; calculated for $C_{29}H_{27}N_7O_4$: C, 64.79; H, 5.06; N, 18.24; found C, 64.85; H, 5.11; N, 18.29.

2.4.4. N^3,N^5-diisonicotinyl-2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5- dicarbohydrazide (4c)

Light-greenish solid; M.P: 259–261 °C; yield: 66%; IR (KBr, cm^{-1}): 3304 (N—H), 3108 (Ar-C—H), 2982 (Ali-C—H), 1669 (C═O, amide), 1453 (Ar-C═C), 1372 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.75 (*s*, 6H, $(CH_3)_2$), 4.49 (*s*, 1H, CH), 6.19 (*bs*, 1H, NH), 7.32 (*d*, 2H, Ar-H), 7.56 (*s*, 1H, Ar-H), 7.92 (*d*, 2H, (Ar-H)), 7.99 (*d*, 2H, (Ar-H)), 8.04 (*s*, 1H, Ar-H), 8.08 (*d*, 2H, (Ar-H)), 8.22 (*s*, 1H, NH), 8.25 (*s*, 1H, NH), 8.27 (*s*, 1H, NH), 8.29 (*s*, 1H, NH), (*d*, 2H, Ar-H), 9.11 (*d*, 4H, (*d*, 2H, Ar-H)); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 17.8 (CH_3), 36.3 (CH), 102.8 (2ArC), 121.0 (ArC), 122.7 (4ArC), 126.7 (ArC), 130.0 (ArC), 131.8 (ArC), 134.8 (ArC), 140.9 (2ArC), 149.0 (ArC), 149.8 (4ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 556.18; found 556.14; calculated for $C_{27}H_{24}N_8O_6$: C, 58.27; H, 4.35; N, 20.13; found C, 58.32; H, 4.31; N, 20.16.

2.4.5. 4-(2-Chlorophenyl)-N^3,N^5-diisonicotinyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbohydrazide (4d)

Light-bluish solid; M.P: 283–285 °C; yield: 74%; IR (KBr, cm^{-1}): 3318 (N—H), 3112 (Ar-C—H), 2956 (Ali-C—H), 1672 (C═O, amide), 1481 (Ar-C═C), 1384 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.72 (*s*, 6H, $(CH_3)_2$), 4.52 (*s*, 1H, CH), 6.22 (*bs*, 1H, NH), 7.02–7.17 (*m*, 4H, Ar-H), 7.92 (*d*, 2H, (Ar-H), 7.98 (*d*, 2H, (Ar-H)), 8.06 (*s*, 1H, NH), 8.12 (*s*, 1H, NH), 8.19 (*s*, 1H, NH), 8.24 (*s*, 1H, NH), 9.12 (*d*, 2H, (Ar-H)), 9.15 (*d*, 2H, (Ar-H)); ^{13}C NMR (125 MHz, DMSO-d6, δ ppm): 16.8 (CH_3), 16.9 (CH_3), 35.9 (CH), 102.8 (2ArC), 118.1 (ArC), 122.7 (4ArC), 124.3 (ArC), 129.6 (ArC), 135.2 (ArC), 140.9 (2ArC), 143.1 (ArC), 148.2 (2ArC), 149.5 (2ArC), 149.8 (4ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 545.15; found 545.21; calculated for $C_{27}H_{24}ClN_7O_4$: C, 59.40; H, 4.43; N, 17.96; found C, 59.46; H, 4.48; N, 17.93.

2.4.6. N^3,N^5-diisonicotinyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarbohydrazide (4e)

Light-yellowish solid; M.P: 262–264 °C; yield: 76%; IR (KBr, cm^{-1}): 3305 (N—H), 3126 (Ar-C—H), 2934 (Ali-C—H), 1669 (C═O, amide), 1471 (Ar-C═C), 1376 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.76 (*s*, 6H, $(CH_3)_2$), 4.49 (*s*, 1H, CH), 6.17 (*bs*, 1H, NH), 7.47 (*d*, 2H, Ar-H), 7.94 (*d*, 2H, Ar-H), 7.97 (*d*, 2H, Ar-H), 8.04 (*d*, 2H, (Ar-H), 8.12 (*s*, 1H, NH), 8.18 (*s*, 1H, NH), 8.22 (*s*, 1H, NH), 8.29 (*s*, 1H, NH), 9.04 (*d*, 2H, (Ar-H)), 9.13 (*d*, 2H, (Ar-H)); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 16.8 (CH_3), 43.9 (CH), 102.8 (2ArC), 122.7 (4ArC), 126.7 (ArC), 127.8 (ArC), 128.7 (ArC), 130.4 (ArC), 134.4 (ArC), 140.8 (2ArC), 143.6 (ArC), 149.5 (2ArC), 149.8 (4ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 556.18; found 556.13; calculated for $C_{27}H_{24}N_8O_6$: C, 58.27; H, 4.35; N, 20.13; found C, 58.32; H, 4.39; N, 20.19.

2.4.7. 4-(3-Chlorophenyl)-N^3,N^5-diisonicotinyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbohydrazide (4f)

Light gray-colored solid; M.P: 284–286 °C; yield 73%; IR (KBr, cm^{-1}): 3312 (N—H), 3118 (Ar-C—H), 2952 (Ali-C—H), 1673 (C═O, amide), 1478 (Ar-C═C), 1362 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.74 (*s*, 6H, $(CH_3)_2$), 4.47 (*s*, 1H, CH), 6.23 (*bs*, 1H, NH), 6.96–7.12 (*m*, 4H, Ar-H), 7.96 (*d*, 2H, Ar-H), 8.01 (*d*, 2H, Ar-H), 8.05 (*s*, 1H, NH), 8.08 (*s*, 1H, NH), 8.13 (*s*, 1H, NH), 8.19 (*s*, 1H, NH), 9.02 (*d*, 2H, Ar-H), 9.09 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 16.8 (CH_3), 44.5 (CH), 102.7 (2ArC), 122.8 (4ArC), 125.9 (ArC), 127.1 (ArC), 128.9 (ArC), 130.0 (ArC), 134.2 (ArC), 140.9 (2ArC), 143.5 (ArC), 149.5 (2ArC), 149.8 (4ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 545.15; found 545.19; calculated for $C_{27}H_{24}ClN_7O_4$: C, 59.40; H, 4.43; N, 17.96; found C, 59.44; H, 4.48; N, 18.02.

2.4.8. 4-(3-Fluorophenyl)-N^3,N^5-diisonicotinyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbohydrazide (4g)

Light-greenish solid; M.P: 297–299 °C; yield 77%;IR (KBr, cm^{-1}): 3318 (N—H), 3124 (Ar-C—H), 2936 (Ali-C—H), 1668 (C═O, amide), 1473 (Ar-C═C), 1380 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.73 (*s*, 6H, $(CH_3)_2$), 4.45 (*s*, 1H, CH), 6.20 (*bs*, 1H, NH), 6.76–7.06 (*m*, 4H, Ar-H), 7.94 (*d*, 2H, Ar-H), 8.02 (*d*, 2H, Ar-H), 8.12 (*s*, 1H, NH), 8.17 (*s*, 1H, NH), 8.24 (*s*, 1H, NH), 8.27 (*s*, 1H, NH), 9.03 (*d*, 2H, Ar-H), 9.11 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d6, δ ppm): 16.7 (CH_3), 16.8 (CH_3), 45.0 (CH), 102.7 (2ArC), 112.4 (ArC), 116.1 (ArC), 122.8 (4ArC), 124.6 (ArC), 130.3 (ArC), 140.8 (2ArC), 143.8 (ArC), 149.5 (2ArC), 149.8 (4ArC), 162.7 (ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 529.18; found 529.24; calculated for $C_{27}H_{24}FN_7O_4$: C, 61.24; H, 4.57; N, 18.52; found C, 61.28; H, 4.63; N, 18.49.

2.4.9. 4(4-Chlorophenyl)-N^3, N^5-diisonicotinyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbohydrazide (4h)

Light-bluish solid; M.P: 281–283 °C; yield 81%; IR (KBr, cm^{-1}): 3304 (N—H), 3118 (Ar-C—H), 2922 (Ali-C—H), 1671 (C═O, amide), 1478 (Ar-C═C), 1374 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.75 (*s*, 6H, $(CH_3)_2$), 4.47 (*s*, 1H, CH), 6.23 (*bs*, 1H, NH), 6.98–7.16 (*m*, 4H, Ar-H), 7.95 (*d*, 2H, Ar-H),

8.04 (*d*, 2H, Ar-H), 8.09 (*s*, 1H, NH), 8.13 (*s*, 1H, NH), 8.17 (*s*, 1H, NH), 8.21 (*s*, 1H, NH), 9.06 (*d*, 2H, Ar-H), 9.13 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 16.8 (CH_3), 44.9 (CH), 102.7 (2ArC), 122.8 (4ArC), 128.7 (2ArC), 130.5 (2ArC), 131.3 (ArC), 140.2 (ArC), 140.9 (2ArC), 149.4 (2ArC), 149.8 (4ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 545.15; found 545.20; calculated for $C_{27}H_{24}ClN_7O_4$: C, 59.40; H, 4.43; N, 17.96; found C, 59.45; H, 4.49; N, 18.01.

2.4.10. 4(4-Fluorophenyl)-N^3,N^5-diisonicotinyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbohydrazide (4i)

Dark-brownish solid; M.P: 306–308 °C; yield: 65%; IR (KBr, cm^{-1}): 3313 (N—H), 3140 (Ar-C—H), 2938 (Ali-C—H), 1668 (C═O, amide), 1484 (Ar-C═C), 1381 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.78 (*s*, 6H, $(CH_3)_2$), 4.44 (*s*, 1H, CH), 6.18 (*bs*, 1H, NH), 6.86–7.06 (*m*, 4H, Ar-H), 7.93 (*d*, 2H, Ar-H), 8.01 (*d*, 2H, Ar-H), 8.08 (*s*, 1H, NH), 8.12 (*s*, 1H, NH), 8.16 (*s*, 1H, NH), 8.19 (*s*, 1H, NH), 9.02 (*d*, 2H, Ar-H), 9.10 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 16.8 (CH_3), 45.0 (CH), 102.8 (2ArC), 115.3 (2ArC), 122.7 (4ArC), 130.6 (2ArC), 137.8 (ArC), 140.8 (2ArC), 149.4 (2ArC), 149.7 (4ArC), 159.8 (ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 529.18; found 529.13; calculated for $C_{27}H_{24}FN_7O_4$: C, 61.24; H, 4.57; N, 18.52; found C, 61.29; H, 4.63; N, 18.47.

2.4.11. N^3,N^5-diisonicotinyl-2,6-dimethyl-4-(pyridine-2-yl)-1,4-dihydropyridine-3,5-dicarbohydrazide (4j)

Light-bluish solid; M.P: 293–295 °C; yield: 73%; IR (KBr, cm^{-1}): 3313 (N—H), 3144 (Ar-C—H), 2952 (Ali-C—H), 1670 (C═O, amide), 1476 (Ar-C═C), 1368 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.74 (*s*, 6H, $(CH_3)_2$), 4.48 (*s*, 1H, CH), 6.22 (*bs*, 1H, NH), 7.27 (*d*, 2H, Ar-H), 7.78 (*s*, 1H, Ar-H), 7.95 (*d*, 2H, Ar-H), 8.02 (*d*, 2H, Ar-H), 8.09 (*s*, 1H, NH), 8.13 (*s*, 1H, NH), 8.15 (*s*, 1H, NH), 8.17 (*s*, 1H, NH), 8.69 (*s*, 1H, Ar-H), 9.02 (*d*, 2H, Ar-H), 9.10 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 16.8 (CH_3), 31.7 (CH), 102.7 (2ArC), 120.9 (ArC), 122.7 (4ArC), 124.1 (ArC), 136.1 (ArC), 140.8 (2ArC), 148.7 (ArC), 149.5 (2ArC), 149.8 (4ArC), 158.5 (ArC), 164.8 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 512.19; found 512.25; calculated for $C_{26}H_{24}N_8O_4$: C, 60.93; H, 4.72; N, 21.86; found C, 60.90; H, 4.76; N, 21.90.

2.4.12. N^3,N^5-diisonicotinyl-2,6-dimethyl-4-(pyridine-3-yl)-1,4-dihydropyridine-3,5-dicarbohydrazide (4k)

Light-yellowish solid; M.P: 273–275 °C; yield: 80%; IR (KBr, cm^{-1}): 3308 (N—H), 3158 (Ar-C—H), 2940 (Ali-C—H), 1668 (C═O, amide), 1456 (Ar-C═C), 1372 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.76 (*s*, 6H, $(CH_3)_2$), 4.44 (*s*, 1H, CH), 6.17 (*bs*, 1H, NH), 7.31 (*s*, 1H, Ar-H), 7.72 (*s*, 1H, Ar-H), 7.93 (*d*, 2H, Ar-H), 8.01 (*d*, 2H, Ar-H), 8.11 (*s*, 1H, NH), 8.15 (*s*, 1H, NH), 8.19 (*s*, 1H, NH), 8.23 (*s*, 1H, NH), 8.59 (*d*, 2H, Ar-H), 9.07 (*d*, 2H, Ar-H), 9.15 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 16.8 (CH_3), 44.9 (CH), 102.8 (2ArC), 122.7 (4ArC), 123.0 (ArC), 132.5 (ArC), 134.3 (ArC), 140.8 (2ArC), 147.4 (ArC), 149.4 (2ArC), 149.8 (4ArC) 150.1 (ArC), 164.9 (2C═O), 165.2 (2C═O); MS (m/z): (M + 1) calculated 512.19; found 512.22; calculated for $C_{26}H_{24}N_8O_4$: C, 60.93; H, 4.72; N, 21.86; found C, 60.99; H, 4.77; N, 21.92.

2.4.13. N^3,N^5-diisonicotinyl-2,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyridine-3,5-dicarbohydrazide (4l)

Light gray-colored solid; M.P: 233–235 °C; yield: 62%; IR (KBr, cm^{-1}): 3298 (N—H), 3184 (Ar-C—H), 2966 (Ali-C—H), 1673 (C═O, amide), 1487 (Ar-C═C), 1378 (C—N); ^{1}H NMR (DMSO-d_6) δ: 1.78 (*s*, 6H, $(CH_3)_2$), 4.47 (*s*, 1H, CH), 6.24 (*bs*, 1H, NH), 7.32 (*d*, 2H, Ar-H), 7.95 (*d*, 2H, Ar-H), 8.03 (*d*, 2H, Ar-H), 8.04 (*s*, 1H, NH), 8.09 (*s*, 1H, NH), 8.12 (*s*, 1H, NH), 8.17 (*s*, 1H, NH), 8.63 (*d*, 2H, Ar-H), 9.04 (*d*, 2H, Ar-H), 9.11 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.7 (CH_3), 16.7 (CH_3), 45.0 (CH), 102.7

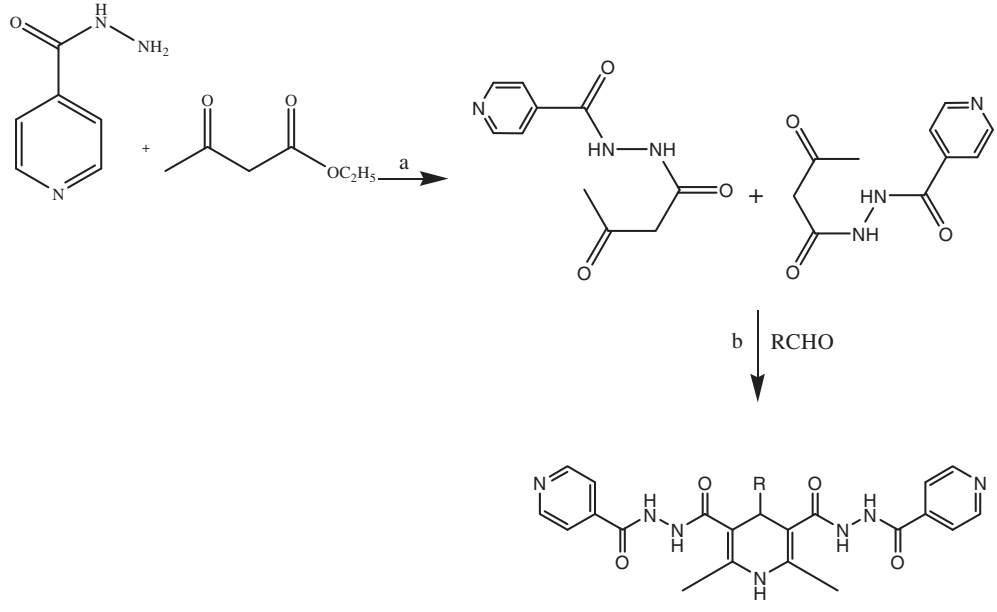


Figure 1 Synthesis of compounds (4a–4m). Reagents and conditions: (a) reflux 3.0 h, 10 ml of ethanol (95%) and potassium tert-butoxide; (b) C_2H_5OH, barium nitrate 25–30% aqueous ammonia solution, and reflux for 11–18 h.

(2ArC), 122.8 (4ArC), 124.2 (2ArC), 140.8 (2ArC), 149.4 (2ArC), 149.8 (4ArC), 149.9 (2ArC), 151.2 (ArC), 164.9 (2C═O), 165.1 (2C═O); MS (m/z): (M + 1) calculated 512.19; found 512.15; calculated for $C_{26}H_{24}N_8O_4$: C, 60.93; H, 4.72; N, 21.86; found C, 60.97; H, 4.74; N, 21.84.

2.4.14. 4-(Furan-2-yl)-N^3,N^5-diisonicotinyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarbohydrazide (4m)

Light gray-colored solid; M.P: 245–247 °C; yield: 64%; IR (KBr, cm^{-1}): 3307 (N─H), 3162 (Ar-C─H), 2978 (Ali-C─H), 1671 (C═O, amide), 1475 (Ar-C═C), 1368 (C─N); ^{1}H NMR (DMSO-d_6) δ: 1.74 (*s*, 6H, $(CH_3)_2$), 4.44 (*s*, 1H, CH), 5.86 (*s*, 1H, Ar-H), 6.20 (*bs*, 1H, NH), 6.20 (*s*, 1H, (Ar-H), 7.23 (*s*, 1H, (Ar-H), 7.96 (*d*, 2H, Ar-H), 8.01 (*d*, 2H, Ar-H), 8.09 (*s*, 1H, NH), 8.13 (*s*, 1H, NH), 8.17 (*s*, 1H, NH), 8.22 (*s*, 1H, NH), 9.03 (*d*, 2H, Ar-H), 9.10 (*d*, 2H, Ar-H); ^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 16.1 (CH_3), 16.2 (CH_3), 32.9 (CH), 102.7 (2ArC), 106.7 (ArC), 110.5 (ArC), 122.7 (4ArC), 140.8 (2ArC), 142.1 (ArC), 149.4 (2ArC), 149.8 (4ArC), 152.4 (ArC), 164.9 (2C═O), 165.1 (2C═O); MS (m/z): (M + 1) calculated 501.17; found 501.12; calculated for $C_{25}H_{23}N_7O_5$: C, 59.87; H, 4.62; N, 19.55; found C, 59.93; H, 4.67; N, 19.60.

2.5. Antimicrobial activity

The *in vitro* antibacterial activities were tested against Gram-positive bacteria *Bacillus subtilis* and Gram-negative

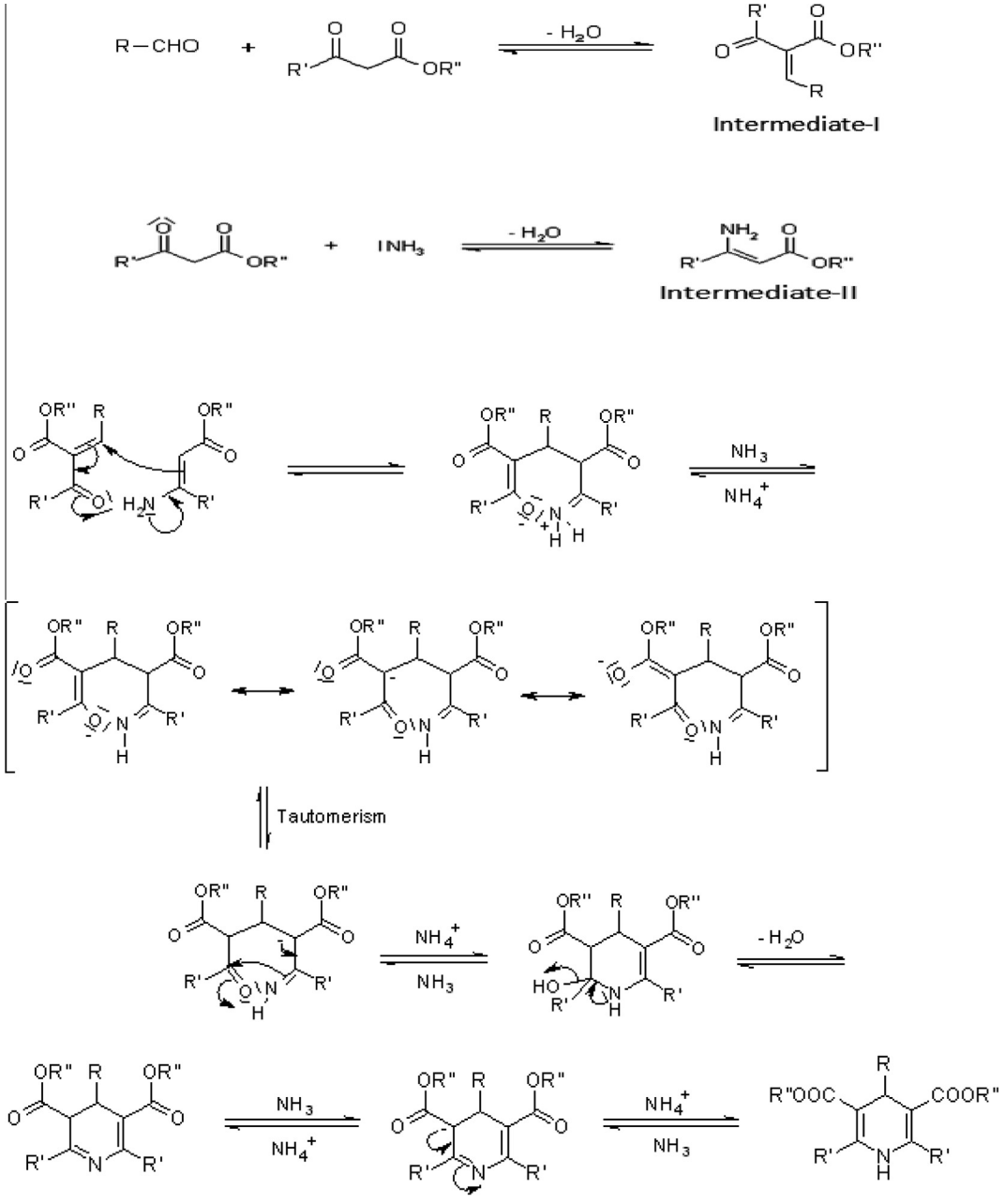


Figure 2 General mechanism of Hantzsch 1,4-dihydropyridines.

bacteria *Escherichia coli* (*E. coli*) by a standard serial dilution method using a stock solution of 100 μg/ml concentrations.[30,31] The double strength nutrient broth was used as culture media and dimethyl sulphoxide (DMSO) was used as solvent control. The stock solutions of the test compounds were serially diluted in test tubes containing 1 ml of sterile medium to get different concentrations and then inoculated with 100 μL of suspension of respective microorganisms in sterile saline. Norfloxacin was used as standard drug. The inoculated test tubes were incubated at 37 ± 1 °C for 24 h.

2.6. In vitro cytotoxicity

Short-term *in vitro* cytotoxicity assay was performed using *Vero cells* (African Monkey kidney cells), according to the standard procedure. Sulforhodamine B (SRB) is a bright-pink amino xanthene dye with two sulfonic groups.[5] Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloroacetic acid fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. After incubation, the solutions in the wells were flicked off and 100 μl of different concentrations (2–500 μg) of compounds were added to the cells and incubated at 37 °C for 3 days in 5% CO_2 atmosphere. The microscopic examinations were performed and observations were recorded every 24 h. After, 72 h, 50% trichloroacetic acid (25 μl) was added to each well and the plates were incubated for 1 h at 4 °C. The supernatant was then removed, and the cells were washed with water, air-dried, and stained, each well with SRB for 30 min. The unbound dye was removed by washing with 1% acetic acid and the plates were air dried. Tris base (10 mM, 100 μl) was added to wells to solubilize the dye. The plates were vigorously shaken for 5 min, and the absorbance was measured using a Microtiter plate reader at 540 nm. The mean absorbance of triplicate was recorded. Mean observance taken from cells grown in the absence of the test compound was taken as 100% cell survival (control). The percentage growth inhibition was calculated using the following formula:

$$\text{Growth inhibition } \% = 100 - [\text{sample absorbance}/\text{control absorbance}] \times 100$$

3. Result and discussion

A series of 13 novel isoniazid cyclocondensed 1,4-pyridines of biological interest were synthesized and evaluated for antimicrobial activity and *in vitro* cytotoxicity, all the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis of their structures.

3.1. Chemistry

Synthesis of 1,4-pyridines by adopting the Hantzsch synthetic protocol involving one-pot multicomponent reaction was performed by following the steps as outlined in Fig. 1. In the first step, ethyl acetoacetate 2 and isoniazid 1 and a catalytic amount of potassium tert-butoxide[8] was taken into a 250 ml RB flask and dissolved in 15 ml of ethanol (95%) reacted under neat conditions resulting in the formation of **N**-(3-oxobutanoyl) isonicotinohydrazide 3 with the yield of 71 percent. The **N**-(3-oxobutanoyl) isonicotinohydrazide 3 was further taken for the Hantzsch condensation reaction by reacting it with appropriate aldehyde, a catalytic amount of barium nitrate and 3 ml of 25–30% aqueous ammonia solution[5] in the presence of 15 ml of ethanol.

The first step in the mechanism of the Hantzsch reaction is the condensation of the β-ketoester with the aldehyde, it produces Intermediate I, this reaction proceeding through Knoevenagel condensation. In the second step β-ketoester reacts with ammonia, it produces enamine Intermediate II.[4] The further condensation between these two intermediates gives the 1,4-dihydropyridine derivative (Fig. 2). The reaction times were found to be 11–18 h. The IR spectra of compounds 4a–m are showing strong absorption bands for the amine group (3286–3318 cm^{-1}), amide group (1664–1673 cm^{-1}), aliphatic C—H stretches (2922–3010 cm^{-1}), aromatic C—H stretching (3081–3184 cm^{-1}), aromatic C=C stretching (1453–1487 cm^{-1}) and C—N (1328–1384 cm^{-1}). The ^{1}H NMR spectrum of compounds 4a–m is showing a two methyl group proton singlet at 1.72–1.78 ppm, pyridine-CH-R proton singlet at 4.44–4.58 ppm, pyridine-NH-proton singlet at 6.16–6.24 ppm, aromatic proton multiplet at 5.86–9.15 ppm and amide (NH) proton singlet at 8.04–8.34 ppm. The ^{13}C NMR spectra, mass spectra and elemental analysis results were within ±0.6% of the theoretical values. Totally, thirteen compounds 4a–m, various substituted 1,4-dihydropyridines, were synthesized with the yield ranging from 56% to 81%. These conditions enable this method to be applicable for the synthesis of 1,4-dihydropyridines based heterocyclic compounds. The present protocol best describes the synthesis of 1,4-dihydropyridines. All the reported 1,4-dihydropyridine compounds were found to be novel and not reported elsewhere.

3.2. Antimicrobial activity

The synthesized compounds were subjected to *in vitro* antimicrobial activity against Gram-positive bacteria *B. subtilis*, Gram-negative bacteria *E. coli*. The motive is to check the antimicrobial activity of the synthesized compounds. The activity is expressed as minimum inhibitory concentration (MIC), the lowest concentration of compound that completely inhibited the growth on the culture.[8] The antimicrobial activity

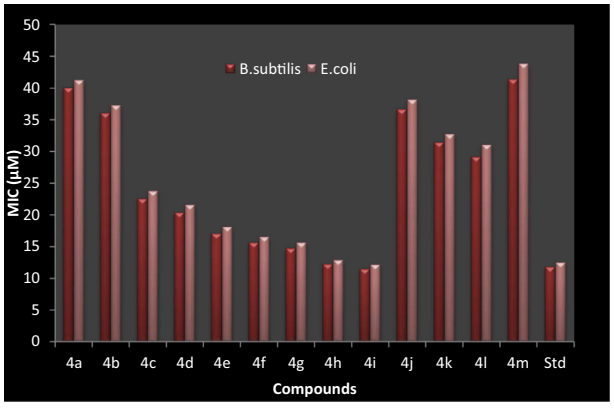
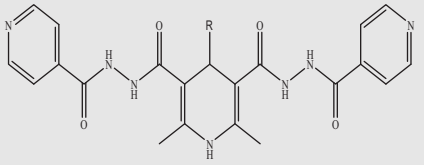
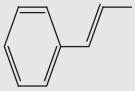
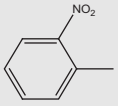
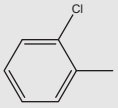
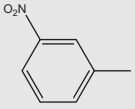
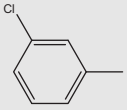
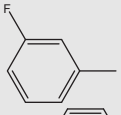
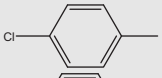
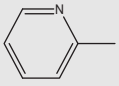
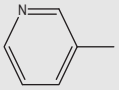
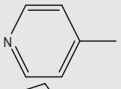
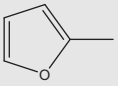


Figure 3 *In vitro* antimicrobial activity of compounds (4a–m) and Norfloxacin (std).

Table 1 Synthesized 1,4-dihydropyridines: *in vitro* antimicrobial activity and cytotoxicity.

General Structure of 1,4-dihydropyridines



S. no.	Compound	R	*B. subtilis* MIC (μM)	*E. coli* MIC (μM)	CTC_{50} (μM) on *Vero Cells*
1	4a		40.1	41.3	138
2	4b		36.1	37.3	110
3	4c		22.6	23.8	90
4	4d		20.4	21.6	69
5	4e		17.1	18.2	53
6	4f		15.7	16.6	46
7	4g		14.8	15.7	39
8	4h		12.3	12.9	33
9	4i		11.5	12.2	27
10	4j		36.7	38.2	90
11	4 k		31.5	32.8	74
12	4l		29.2	31.1	68
13	4m		41.4	43.9	83
14	Norfloxacin	Standard	11.8	12.5	–
15	Tamoxifen	Standard	–	–	32

of the synthesized compounds is shown in (Fig. 3 and Table 1). The data listed in (Fig. 3 and Table 1) clearly showed that most of the designed compounds exhibited good to moderate, or high antimicrobial activities toward the Gram-positive *B. subtilis* and Gram-negative *E. coli*. All the 1, 4-dihydropyridines were potent antimicrobial agents, with an

MIC value ranging from micro molar to submicromolar. Especially, compound 4i showed the best antimicrobial activity of all the 1,4-dihydropyridine derivatives, with an MIC value of 11.5 μM and 12.2 μM.

3.3. In vitro cytotoxicity

The synthesized compounds were subjected to *in vitro* cytotoxicity assay against *Vero cells*. The assay was performed by the Sulforhodamine B (SRB) method. The *in vitro* cytotoxicity by the SRB method was measured in μM. The motive for us to check the cytotoxicity of the synthesized compounds was, some reports that have claimed significant cytotoxicity for some of the 1,4-dihydropyridines.[5] The 1,4-dihydropyridines act primarily by impairing cell reproductive integrity. Thus, affected cells may remain alive and may continue to perform many of their functions but are unable to reproduce successfully.[15] The synthesis of DNA, RNA and other cell constituents may continue at normal rates. However, the cell either fails to divide, forming a giant cell, commences a division that cannot be completed, or divides unsuccessfully, resulting in chromosomes being unequally shared between the daughter cells or being damaged in some way; these chromosomal lesions may eventually cause cell death.[16] The results of the *in vitro* cytotoxicity study indicate that the compounds 4e, 4f, 4g, 4h and 4i enhance the cytotoxicity against *Vero cells*. Compound 4i happens to be the most potent compound among all the 1,4-dihydropyridines studied here by enhancing the cytotoxicity with a CTC_{50} value of 27 μM. The rest of the compounds exhibit weak to moderate cytotoxicity (Fig. 4 and Table 1).

3.4. Structural activity relationship

Analyzing the biological activities of the synthesized compounds, the following structure activity relationships (SAR) were obtained. The third and fifth positions of 1,4-dihydropyridines containing the isoniazid moiety seem to be the hydrophobic trunk, which in turn is connected to the 1,4-dihydropyridines along with the amide bond, also seems to be important structural components of the compounds 4a–m to exhibit significant antimicrobial activity and cytotoxicity. This indicates the necessity of amide groups nearer to the

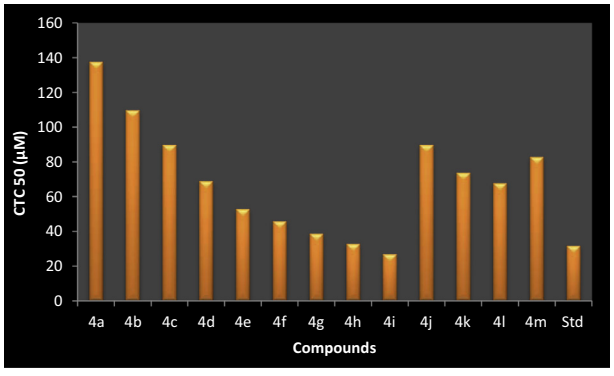


Figure 4 *In vitro* cytotoxicity of compounds (4a–m) and Tamoxifen (std).

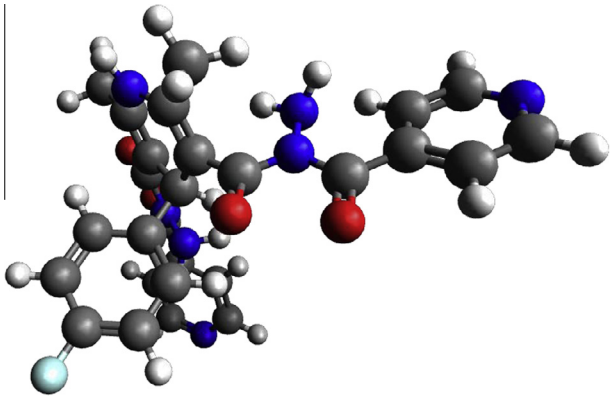


Figure 5 Molecular structure of compound 4i.

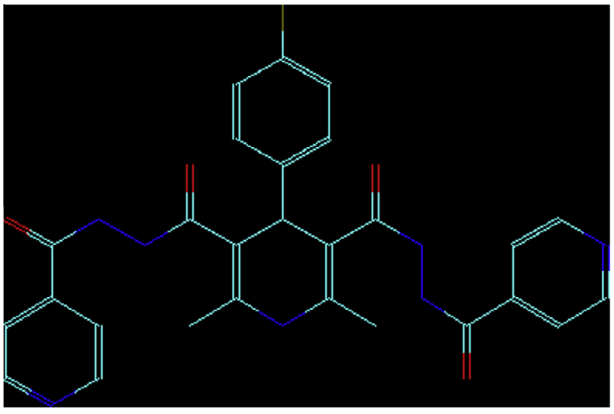


Figure 6 3D-structure of compound 4i.

1,4-dihydropyridine head. The isonicotinoyl hydrazone ring system is found to be the pharmacophore structural part as allmost all compounds possessed this substructure. The difference in bioactivity of 1,4-dihydropyridines could be related to the substituent's at the fourth position of aryl ring.[6] Compounds 4a–i contain a strong electron withdrawing group substituted phenyl ring at the fourth position of 1,4-dihydropyridines for potent activity, because it decreases the electron density in the ring, due to the inductive effect (Figs. 5 and 6). The fluoride and chloride substitution at fourth phenyl ring showed potent action because of strong electron withdrawing nature.[8,11] The substitution of the fluro and the chloro group at the 3rd position of the phenyl ring showed potent action when compared with the nitro group substituted phenyl ring. The aryl substituent at the fourth position of 1,4-hydropyridines order of potency has been found to be 4-fluoro > 4-chloro > 3-fluoro > 4-pyridyl > 3-chloro > 3-nitro > 2-chloro > 2-nitro > H. Heteroaryl substituted compounds at the fourth position showed moderate antimicrobial activity and cytototoxicity.[14] The decreasing ring size from the six membered ring to the five member furan (4 m) is found to reduce the activity. Among the compounds reported herein, compound 4i showed potent antimicrobial activity and cytotoxicity because the fluoride substituted aromatic ring present at the fourth position of 1,4-dihydropyridines enhances the compounds (Figs. 5, 6 and Table 1).

4. Conclusion

A series of novel 1,4-dihydropyridines of biological interest were synthesized and analyzed for their structures. The libraries of compounds were prepared by using barium nitrate as an efficient catalyst. The importance of substitutions at the fourth positions of 1,4-dihydropyridines was studied toward the antimicrobial activity and cytotoxicity. The antimicrobial activity and cytotoxicity data revealed that the all synthesized compounds proved to be active against Gram-positive bacteria *B. subtilis*, Gram-negative bacteria *E. coli* and *Vero cells*. Almost all of the titled compounds exhibited weak, moderate, or high antimicrobial activity and cytotoxicity. Compound 4i is arguably the most potent when compared with Norfloxacin and Tamoxifen and our present study makes it an interesting compound when compared to the current therapeutic agents and is considered as the candidate to investigate further for the same.

Conflict of interest

None

Acknowledgment

The authors wish to thank the Sunrise University for research support. Also, thanks are given to the Molecules Drugs and Research Laboratory, Chennai, India for *in-vitro* antimicrobial activity and cytotoxicity studies.

References

1. Adam MP, Aleksandra S, Beata S, Grzegorz M, Pawe1 S. Synthesis and evaluation of antimicrobial activity of hydrazones derived from 3-oxido-1H-imidazole-4-carbohydrazides. *Eur J Med Chem* 2013;**64**:389–95.
2. Leyla Y, Yusuf O, Zafer AK, Yagmur T, Hulya K. Synthesis and antimicrobial activity of some new hydrazone-bridged thiazole–pyrrole derivatives. *J Enzyme Inhib Med Chem* 2013;**28**:830–5.
3. Sandip NG, Vijay LM, Kisan MK, Murlidhar SS, Dhananjay VM. Synthesis and biological evaluation of novel 2,4,6-triazine derivatives as antimicrobial agents. *Bioorg Med Chem Lett* 2012;**22**:5075–7.
4. Hantzsch A. Uber die synthese pyridinartiger verbindungen aus acetessigather und aldehydammoniak Justus Liebigs. *Ann Chim* 1882;**215**:1–15.
5. Prashantha KB, Pankaj M, Karthikeyan E, Ankur B, Suja, Pottekad V. Synthesis of novel Hantzsch dihydropyridines and Biginelli dihydropyrimidines of biological interest: a 3D-QSAR study on their cytotoxicity. *Med Chem Res* 2010;**19**:344–63.
6. Arkadij S, Maurice CF, Brigita V, Brigita C, Natalija M, Gunars G, Aede G. An efficient chemoenzymatic approach to enantiomerically pure 4-[2-(difluoromethoxy)phenyl] substituted 1,4-dihydropyridine-3,5-dicarboxylates. *Tetrahedron Asymmetry* 2001;**12**:3251–6.
7. Claudius C, Jorg W, Martin K, Christiane B, Marianne S, Josef L, Hermann L, Andreas H. Novel structure–activity relationships and selectivity profiling of cage dimeric 1,4-dihydropyridines as multidrug resistance (MDR) modulators. *Bioorg Med Chem* 2010;**18**:4983–90.
8. Kalam S, Darna B, Garlapati A, Vanga MR. Synthesis, antibacterial and antimycobacterial activities of some new 4-aryl/heteroaryl-2,6-dimethyl-3,5-bis-N-(aryl)-carbamoyl-1,4-dihydropyridines. *Eur J Med Chem* 2011;**46**:1564–71.
9. Ravindra MK, Umesh BK, Pankaj KB, Abhinav K, Appalanaidu LD, Tulshiram LD, Sanjay KB. Synthesis and evaluation of novel triazoles and mannich bases functionalized 1,4-dihydropyridine as angiotensin converting enzyme (ACE) inhibitors. *Bioorg Med Chem* 2014;**22**:5824–30.
10. Prasanthi G, Prasad KV, Bharathi K. Synthesis, anticonvulsant activity and molecular properties prediction of dialkyl 1-(di(ethoxycarbonyl)methyl)-2,6-dimethyl-4-substituted-1,4-dihydropyridine-3,5-dicarboxylates. *Eur J Med Chem* 2014;**73**:97–104.
11. Shrikanth U, Ramakrishna S, Rajesh R, Airody VA. Synthesis, anticonvulsant and anti-inflammatory studies of new 1,4-dihydropyridin-4-yl-phenoxyacetohydrazones. *Eur J Med Chem* 2013;**70**:341–9.
12. Vijesh AM, Arun MI, Peethambar SK, Shivananda KN, Arulmoli AI, Nishitha AI. Hantzsch reaction: synthesis and characterization of some new 1,4-dihydropyridine derivatives as potent antimicrobial and antioxidant agents. *Eur J Med Chem* 2011;**46**:5591–7.
13. Atul K, Ram AM, Siddharth S, Mukesh K, Gitika B. Synthesis and biological evaluation of N-aryl-1,4-dihydropyridines as novel antidyslipidemic and antioxidant agents. *Eur J Med Chem* 2010;**46**:501–9.
14. Ashish R, Vijay K, Abhay B, Hardevsinh V, Shailesh T, Dhairya S, Anamik S, Evans C. Synthesis and 3D-QSAR study of 1,4-dihydropyridine derivatives as MDR cancer reverters. *Eur J Med Chem* 2014;**74**:375–87.
15. Hebat AA, Wael AS, Nahed MF. Synthesis and antitumor activity of new dihydropyridine thioglycosides and their corresponding dehydrogenated forms. *Eur J Med Chem* 2010;**45**:973–82.
16. Shigeyuki T, Hiromasa O, Noriaki G, Mayumi I, Tosiki M, Akira N, Seiji N, Michihiko K. Synthesis and structure ± activity analysis of novel dihydropyridine derivatives to overcome multidrug resistance. *Bioorg Med Chem Lett* 2001;**11**:275–7.
17. Sirisha K, Maddela CS, Kulandaivelu U, Porika M, Abbagani S, Garlapati A, Vanga MR. Molecular docking studies and *in vitro* screening of new dihydropyridine derivatives as human MRP1 inhibitors. *Bioorg Med Chem* 2011;**19**:3249–54.
18. Surendra KR, Idhayadhulla A, Jamal AN, Selvin J. Synthesis and anticoagulant activity of a new series of 1,4-dihydropyridine derivatives. *Eur J Med Chem* 2011;**46**:804–10.
19. Amit RT, Dipti KD, Bipin HD, Vipul BK, Vimal RB, Viresh HS. Synthesis and biological evaluation of some novel N-aryl-1,4 -dihydropyridines as potential antitubercular agents. *Bioorg Med Chem Lett* 2011;**21**:5181–3.
20. Mehdi K, Najmeh E, Katayoun J, Abdolvahab A, Murthy YL, Abdul R, Taraka RM, Jeson PJ, Aruna LK. Design, solvent free synthesis, and antimicrobial evaluation of 1,4 dihydropyridines. *Bioorg Med Chem Lett* 2012;**22**:6016–23.
21. Bahman P, Jalal M, Ramin M. Synthesis and biological evaluation of some new 1,4-dihydropyridines containing different ester substitute and diethyl carbamoyl group as anti-tubercular agents. *Bioorg Med Chem* 2009;**17**:1579–86.
22. Andreas H, Andreas B, Hauke L. Synthesis and biological evaluation of first *N*-alkyl syn dimeric 4-aryl-1,4-dihydropyridines as competitive HIV-1 protease inhibitors. *Eur J Med Chem* 2001;**36**:367–74.
23. Bangle Z, Wei H, Xin S, Menglei H, Qiuju H, Siyuan Z. Synthesis and biological activity of the calcium modulator (R) and (S)-3-methyl 5-pentyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. *Bioorg Med Chem Lett* 2010;**20**:805–8.
24. Rafael L, Cristobal R, Jose MC, Manuela GL, Antonio GG, Mercedes V. Synthesis of 6-amino-1,4-dihydropyridines that prevent calcium overload and neuronal death. *Eur J Med Chem* 2008;**43**:668–74.
25. Jeffrey TN, Carlos AV, Edward EK. Hantzsch 1,4-dihydropyridine containing a diazen-1-ium-1,2-diolate nitric oxide donor moiety to study calcium channel antagonist structure–activity

relationships and nitric oxide release. *Bioorg Med Chem* 2005;**13**:1725–38.
26. Ramin M, Katayoun J, Hasti S, Bahram H. Synthesis, study of 3D structures, and pharmacological activities of lipophilic nitroimidazolyl-1,4-dihydropyridines as calcium channel antagonist. *Bioorg Med Chem* 2006;**14**:4842–9.
27. Gilani SJ, Khan SA, Alam O, Siddiqui N. Synthesis and *in vitro* antimicrobial evaluation of condensed heterocyclic 6-substituted 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives of isoniazid. *Act Pol Pharm* 2011;**68**:205–11.
28. Prasad D, Kiranmayi V, Venkateswara SK, Ejazuddin MK, Manjula S, Subhash P. Synthesis, characterization, molecular docking and anti-tubercular activity of plumbagin–isoniazid analog and its β-cyclodextrin conjugate. *Bioorg Med Chem Lett* 2014;**24**:5070–5.
29. Asif H. Amide derivatives of sulfonamides and isoniazid: synthesis and biological evaluation. *Act Pol Pharm* 2009;**66**:513–21.
30. Vasudeva R, Rajendra Y, Girijasankar G, Pradeepsagar G. Synthesis, characterization and *in vitro* biological evaluation of some novel diarylsulfonylureas as potential cytotoxic and antimicrobial agents. *Bioorg Med Chem Lett* 2012;**22**:1031–5.
31. Zhu B, Marinelli BA, Goldschmidt R. Synthesis and antibacterial activity of 7-(1,2,3,4-tetrahydropyrrolo [1,2-a]-pyrazin-7-yl) quinolones. *Bioorg Med Chem Lett* 2009;**19**:4933–6.