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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

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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

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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