Synthesis, Antifungal and Toxicity Screening of Newer Isoniazid Derivatives

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Abstract

A series ofisonicotinic acid hydrazide (INH) incorporated derivatives of thiazolidin-4-one (2a-h, 3a-h), azetidin-2-one (4a-h) and 1,3,4-oxadiazole (5a-h) were synthesized in satisfactory yield and pharmacologically evaluated for their in vitro antifungal activity. All the synthesized compounds were in good agreement with elemental and spectral data. A majority of the tested compounds showed good to moderate antifungal activity against all tested pathogenic fungal strains. To evaluate the toxicity of the compounds on liver, estimation of enzymes was also carried out.

Keywords: Thiazolidin-4-one; Azetidin-2-one; 1,3,4-oxadiazole; Isoniazid; Antifungal activity; Enzyme estimation

Introduction

Antimicrobial agents are those inhibitory chemicals which are employed to kill microorganisms or prevent their growth. Infectious diseases account for approximately one-half of all deaths in tropical countries. Although deaths from bacterial and fungal infections have dropped in the developed world, these are still major causes of death in the developing world [1]. In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants) [2]. Antimicrobials reduce or completely block the growth and multiplication of bacteria. This has made them unique for the control of deadly infectious diseases caused by a variety of pathogens. They have transformed our ability to treat infectious diseases such as pneumonia, meningitis, tuberculosis, malaria and AIDS [3]. Literature survey revealed that thiazolidin-4-ones are a class of heterocycles which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities including antimicrobial [4], anti-inflammatory, analgesic, antitubercular and anti diabetic [5-8]. Similarly, the azetidin-2-one derivatives have been reported to possess a wide range of biological activities like antibacterial, antifungal, anti-inflammatory, anticonvulsant, anticance and antitubercular [9-14]. In addition, 1,3,4-oxadiazoles are a class of heterocycles which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities including antimicrobial, anti-inflammatory and analgesic [15-17]. In the design of new compounds, development of hybrid molecules through the combination of different pharmacophores in one structure may lead to compounds with increased antifungal activity. In view of the above mentioned facts and in continuation of our interest in the synthesis of heterocycles containing isoniazid moiety, we report herein the synthesis and antifungal evaluation of some novel structural hybrids incorporating both the isoniazid moiety with thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole ring systems through different linkages. Further, Enzyme estimation was also carried out to assess the toxicity effects of the compounds on liver.

Experimental

All the solvents were of AR grade and were obtained from Merck, CDH and S.D. Fine chemicals. Melting points were determined in open capillary tubes and are uncorrected. All the compounds were subjected to elemental analysis (CHN) and the measured values agreed within ± 0.4% with the calculated ones. Thin layer chromatography was performed on silica gel G (Merck). The spots were developed in an iodine chamber and visualized with an ultraviolet lamp. The solvent systems used were benzene:acetone (8:2, v/v) and toluene:acetic acid:formic acid (5:4:1, v/v). Ashless Whatman No. 1 filter paper was used for vacuum filtration. The IR spectra were recorded in KBr pellets on a (BIO-RAD FTS 135) WIN-IR spectrophotometer. The FAB mass spectra of all the compounds were recorded on a JEOL SX102/DA-600 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. The 1H-NMR spectra were recorded on a Bruker model DPX 300 FT-NMR spectrometer in CDCl3 using tetramethylsilane (Me4Si, TMS) as an internal standard. The chemical shifts are reported in the δ ppm scale [18].

General procedure for the synthesis of (E)-N’-(substitutedbenzylidene)isonicotinohydrazide (1a-h)

To an equimolar methanolic solution of isonicotinic acid hydrazide (0.1mol) and substituted benzaldehyde (0.1mol), a few drops of glacial acetic acid were added. The mixture was then refluxed on water bath for 5-6 h. It was then allowed to cool and poured into crushed ice. Recrystallisation of the dried compounds from methanol yielded compounds 1a-h.

(E)-N’-(2-Chlorobenzylidene)isonicotinohydrazide (1a): Yield: 90%; m.p.184-186°C. Anal. Calcd. for C12H9N2OCl (MW 259.69): C, 56.50; H, 3.42; N,16.18%. Found: C, 56.10; H, 3.46; N, 16.16%. IR (KBr, cm⁻¹): 3300 (N-H stretching), 1680 (C=O stretching of carbonyl), 1600 (~C=CH-C benzene); 1540 (N=CH stretch). 

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(E)-N'-(4-Chlorobenzylidene)isonicotinohydrazide (1b): Yield: 90%; m.p.192-194°C. Anal. Calcd. for C14H10N3O2 (MW 270.24): C, 60.12; H, 4.60; N, 37.28. Found: C, 60.11; H, 4.60; N, 37.27. IR (KBr, cm-1): 3317 (N-H stretching), 1688 (C=O stretching), 1617 (-N=CH-Ar stretching of aromatic ring), 822 (C-N stretching). 1H-NMR (300 MHz, CDCl3, δ/ppm): 7.71, 8.64 (4H, m, Py), 7.6 (1H, s, -N=CH), 7.06-7.11 (4H, m, J = 9 Hz, aromatic). 3.50 (3H, s, OCH3). MS (m/z): 241 [M+].

(E)-N'-(2-Hydroxybenzylidene)isonicotinohydrazide (1c): Yield: 95%; m.p.198-202°C. Anal. Calcd. for C15H12N3O3S (MW 315.07): C, 57.13; H, 4.16; N, 13.33%. Found: C, 57.11; H, 4.14; N, 13.31%. IR (KBr, cm-1): 3318 (N-H stretching), 1643 (C=O stretching). 1H-NMR (300 MHz, CDCl3, δ/ppm): 7.72-7.80 (4H, m, aromatic), 7.12-7.16 (4H, m, J = 9 Hz, aromatic). 3.50 (3H, s, OCH3). MS (m/z): 243 [M+].

(E)-N'-(4-Fluorobenzylidene)isonicotinohydrazide (1f): Yield: 95%; m.p.198-202°C. Anal. Calcd. for C15H12N3O3S (MW 329.37): C, 58.34; H, 4.59; N, 12.76%. Found: C, 58.30; H, 4.56; N, 12.81%. IR (KBr, cm-1): 3332 (N-H stretching), 1632 (C=O stretching), 1662 (C=N). 1H-NMR (300 MHz, CDCl3, δ/ppm): 9.10 (1H, s, CONH-), 7.72-8.64 (4H, m, Py), 7.10-7.13 (4H, m, J = 9 Hz, aromatic). 3.50 (2H, s, CH2). MS (m/z): 333 [M+].

General procedure for the synthesis of N-(2-(substituted phenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2a-h)

A mixture of 1 (0.01 mol) and thioglycollic acid (0.01 mol) was heated on an oil-bath at 120-125°C for 12h. The reaction mixture was cooled and treated with 10% sodium bicarbonate solution. The product was isolated and recrystallised from methanol-dioxane (4:1) to give compounds 2a-h.

N-(2-(3-Hydroxyphenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2a): Yield: 85%; m.p. 198-200°C. Anal. Calcd. for C16H15N3O3S (MW 333.79): C, 53.97; H, 3.62; N, 12.59%. Found: C, 53.94; H, 3.60; N, 12.57%. IR (KBr, cm-1): 3300 (N-H stretching), 1706 (C=O stretching of carbonyl), 1647 (C=O stretching of carbonyl), 1617 (C=O stretching of carbonyl), 820 (C-I stretching of chlorine), 700 (C-S-C). 1H-NMR (300 MHz, CDCl3, δ/ppm): 9.18 (1H, s, CONH-), 7.74-8.64 (4H, m, Py), 7.20 (1H, s, CH=), 7.12-7.15 (4H, aromatic, 3.50 (2H, s, CH2). MS (m/z): 259 [M+].

N-(2-(2-Chlorophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2b): Yield: 85%; m.p. 200-204°C. Anal. Calcd. for C15H12ClN3O2S (MW 332.20): C, 51.26; H, 2.90; N, 12.95%. Found: C, 51.20; H, 2.88; N, 12.98%. IR (KBr, cm-1): 3305 (N-H stretching), 1701 (C=O thiazolidinone), 1646 (C=O stretching of carbonyl), 1617 (C=O stretching of carbonyl), 820 (C-I stretching of chlorine), 700 (C-S-C). 1H-NMR (300 MHz, CDCl3, δ/ppm): 9.18 (1H, s, CONH-), 7.74-8.64 (4H, m, Py), 7.20 (1H, s, CH=), 7.12-7.15 (4H, aromatic, 3.50 (2H, s, CH2). MS (m/z): 241 [M+].

N-(2-(2-Hydroxyphenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2d): Yield: 85%; m.p. 204-206°C. Anal. Calcd. for C15H13N3O3S (MW 315.07): C, 57.13; H, 4.16; N, 13.33%. Found: C, 57.11; H, 4.14; N, 13.31%. IR (KBr, cm-1): 3318 (N-H stretching), 1716 (C=O thiazolidinone), 1644 (C=O stretching of carbonyl), 1615 (C=O stretching of carbonyl), 820 (C-I stretching of chlorine), 700 (C-S-C). 1H-NMR (300 MHz, CDCl3, δ/ppm): 9.14 (1H, s, CONH-), 7.74-8.68 (4H, m, Py), 7.20 (1H, s, CH=), 7.12-7.15 (4H, aromatic, 3.50 (2H, s, CH2). MS (m/z): 237 [M+].
(2H, s, CH₂). 1^3^C-NMR (100 MHz, CDCl₃, δ/ppm): 168.8, 163.7, 159.0, 149.7, 121.7, 140.8, 131.5, 129.7, 121.7, 114.2, 64.3, 55.8, 35.6. MS (m/z): 329 [M⁺].

N-(2-(4-Fluorophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2f): Yield: 65%; m.p. 232-234°C. Anal. Calcld. for C₂₀H₁₈N₄O₆S (MW 391.83): C, 50.78; H, 4.11; N, 10.83% (IR (KBr, cm⁻¹): 3320 (N-H stretching), 1720 (C=O thiazolidinone), 1670 (C=O stretching of carbonyl), 1634 (C=N), 1580 (C=C), 711 (C-S-C). 1H-NMR (300 MHz, CDCl₃, δ/ppm): 10.03 (1H, s, COOH), 9.40 (1H, s, CONH⁻), 7.70 (1H, s, N-CH-), 7.63, 8.55 (4H, m, Ar-H), 6.22-6.18 (4H, m, Ar-H), 5.91 (1H, s, -S-CH-Ar). 1^3^C-NMR (100 MHz, CDCl₃, δ/ppm): 175.3, 173.3, 163.7, 161.3, 149.7, 140.8, 134.8, 130.3, 121.7, 114.2, 61.8, 55.8, 47.5, 39.2. MS (m/z): 387 [M⁺].

2-(3-Hydroxyphenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3e): Yield: 65%; m.p. 220-222°C. Anal. Calcld. for C₂₀H₁₈N₄O₆S (MW 391.83): C, 50.78; H, 4.11; N, 10.83% (IR (KBr, cm⁻¹): 3320 (N-H stretching), 1720 (C=O thiazolidinone), 1670 (C=O stretching of carbonyl), 1634 (C=N), 1580 (C=C), 711 (C-S-C). 1H-NMR (300 MHz, CDCl₃, δ/ppm): 10.03 (1H, s, COOH), 9.40 (1H, s, CONH⁻), 7.70 (1H, s, N-CH-), 7.63, 8.55 (4H, m, Ar-H), 6.22-6.18 (4H, m, Ar-H), 5.91 (1H, s, -S-CH-Ar). 1^3^C-NMR (100 MHz, CDCl₃, δ/ppm): 175.3, 173.3, 163.7, 161.3, 149.7, 140.8, 134.8, 130.3, 121.7, 114.2, 61.8, 55.8, 47.5, 39.2. MS (m/z): 373 [M⁺].

2-(2-Fluorophenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3b): Yield: 85%; m.p. 216-218°C. Anal. Calcld. for C₂₀H₁₈N₄O₆S (MW 391.83): C, 50.78; H, 4.11; N, 10.83% (IR (KBr, cm⁻¹): 3320 (N-H stretching), 1720 (C=O thiazolidinone), 1670 (C=O stretching of carbonyl), 1634 (C=N), 1580 (C=C), 711 (C-S-C). 1H-NMR (300 MHz, CDCl₃, δ/ppm): 10.03 (1H, s, COOH), 9.40 (1H, s, CONH⁻), 7.63, 8.31 (4H, m, Ar-H), 6.24-6.20 (4H, m, Ar-H), 5.91 (1H, s, -S-CH-Ar). 1^3^C-NMR (100 MHz, CDCl₃, δ/ppm): 175.3, 173.3, 163.7, 149.0, 149.7, 140.8, 133.4, 134.7, 129.6, 128.0, 124.8, 121.7, 57.2, 47.5, 39.2. MS (m/z): 402 [M⁺].

2-(2-Chlorophenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3b): Yield: 85%; m.p. 216-218°C. Anal. Calcld. for C₂₀H₁₈N₄O₆S (MW 391.83): C, 50.78; H, 4.11; N, 10.83% (IR (KBr, cm⁻¹): 3320 (N-H stretching), 1720 (C=O thiazolidinone), 1670 (C=O stretching of carbonyl), 1634 (C=N), 1580 (C=C), 711 (C-S-C). 1H-NMR (300 MHz, CDCl₃, δ/ppm): 10.03 (1H, s, COOH), 9.40 (1H, s, CONH⁻), 7.63, 8.31 (4H, m, Ar-H), 6.24-6.20 (4H, m, Ar-H), 5.91 (1H, s, -S-CH-Ar). 1^3^C-NMR (100 MHz, CDCl₃, δ/ppm): 175.3, 173.3, 163.7, 149.0, 149.7, 140.8, 133.4, 134.7, 129.6, 128.0, 124.8, 121.7, 57.2, 47.5, 39.2. MS (m/z): 402 [M⁺].

2-(2-Chlorophenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3b): Yield: 85%; m.p. 216-218°C. Anal. Calcld. for C₂₀H₁₈N₄O₆S (MW 391.83): C, 50.78; H, 4.11; N, 10.83% (IR (KBr, cm⁻¹): 3320 (N-H stretching), 1720 (C=O thiazolidinone), 1670 (C=O stretching of carbonyl), 1634 (C=N), 1580 (C=C), 711 (C-S-C). 1H-NMR (300 MHz, CDCl₃, δ/ppm): 10.03 (1H, s, COOH), 9.40 (1H, s, CONH⁻), 7.63, 8.31 (4H, m, Ar-H), 6.24-6.20 (4H, m, Ar-H), 5.91 (1H, s, -S-CH-Ar). 1^3^C-NMR (100 MHz, CDCl₃, δ/ppm): 175.3, 173.3, 163.7, 149.0, 149.7, 140.8, 133.4, 134.7, 129.6, 128.0, 124.8, 121.7, 57.2, 47.5, 39.2. MS (m/z): 402 [M⁺].

General procedure for the synthesis of 2-(2-substitutedphenyl)-3-(isonicotinamido)-4-oxothiazolidin-5-yl)acetic acid (3a-h)

A mixture of 1 (0.01mol) and thiolactic acid (0.01mol) was heated on an oil-bath at 120-125°C for 12h. The reaction mixture was cooled and treated with 10% sodium bicarbonate solution. The product was isolated and recrystallised from methanol-dioxane (4:1) to give compounds 3a-h.

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6.61 (4H, m, Ar-H). 13C-NMR (100 MHz, CDCl₃, δ/ppm): 163.7, 163.5, 9.80 (1H, s, CONH-), 7.64-8.31 (4H, m, Py), 7.40 (1H, s, N-CH-), 6.60-6.61 (4H, m, aromatic). 13C-NMR (100 MHz, CDCl₃, δ/ppm): 163.7, 163.5, 156.8, 149.7, 144.9, 140.8, 129.9, 121.7, 113.9, 112.6, 67.7, 64.1. MS (m/z): 317[M⁺].

N-(3-Chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl) isonicotinamide (4e): Yield: 55%; m.p. 344-346 °C. Anal. Calcld. for C₁₅H₁₁ClN₃O₄ (MW 319.72): C, 51.96; H, 3.20; N, 16.14%. Found: C, 51.94; H, 3.19; N, 16.14%. IR (KBr, cm⁻¹): 3268 (N-H stretching), 1740 (C=O β-lactam ring), 1662 (C=O stretching of carbonyl), 1465 (N=C). ¹H-NMR (300 MHz, CDCl₃, δ/ppm): 9.40 (1H, s, CONH-), 7.65, 8.34 (4H, m, Py), 7.30 (1H, s, N-CH₂), 6.64-6.66 (4H, m, Ar-H). ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 163.7, 163.5, 149.7, 140.8, 133.0, 129.2, 121.7, 112.7, 63.1, 62.8. MS (m/z): 346[M⁺].

N-(3-Chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl) isonicotinamide (4f): Yield: 65%; m.p. 360-362°C. Anal. Calcld. for C₁₅H₁₁ClFN₃O₂ (MW 319.72): C, 56.35; H, 3.47; N, 13.14%. Found: C, 56.33; H, 3.46; N, 13.12%. IR (KBr, cm⁻¹): 3264 (N-H stretching), 1747 (C=O β-lactam ring), 1662 (C=O stretching of carbonyl), 1614 (C=N). ¹H-NMR (300 MHz, CDCl₃, δ/ppm): 9.40 (1H, s, CONH-), 7.65, 8.34 (4H, m, Py), 7.30 (1H, s, N-CH₂), 6.64-6.66 (4H, Ar-H). ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 163.7, 163.5, 149.7, 140.8, 133.0, 129.2, 121.7, 112.7, 63.1, 62.8. MS (m/z): 344[M⁺].
Antifungal activity

Antifungal activity of the synthesized compounds were determined in vitro by using serial plate dilution method [19, 20] against C. albicans (ATCC 2901), A. niger (MTCC 281), A. flavus (MTCC 277), M. purpureus (MTCC 369) and P. citrinum (NCIM 768) at 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL and 6.25 μg/mL concentrations, respectively, in the nutrient agar media. Standard antibiotic ketoconazole was used as reference drug at 25 μg/mL, 12.5 μg/mL and 6.25 μg/mL. Solutions of required concentrations of test compounds were prepared by dissolving the compounds in DMSO. The minimum inhibitory concentration (MIC) obtained for the test compounds and standard drug are reported in Table 1. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compounds that inhibited visible growth of microorganisms on the plate.

Animals

Male albino mice (Swiss, 18-25 gm) were used as experimental animals. The test compounds were suspended in polyethylene glycol (PEG). The animals were maintained on an adequate diet and allowed free access to food and water except during the short time they were removed from cages for testing. The animals were maintained at room temperature (25-30°C). All the experimental protocols were carried out with the permission from Institutional Animal Ethics Committee (IAEC). Animals were obtained from Central Animal House Facility, Hamdard University, New Delhi-110062, India. Registration number and date of registration of Animal House Facility (173/CPCSEA, 28, JAN-2000).

Assessment of liver function

Liver functions such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assessed by a reported method [21]. The alkaline phosphatase was also measured according to the reported procedures [22,23]. All data are recorded in Table 2.

Results and Discussion

Chemistry

The key intermediates used in the synthesis of thiazolidin-4-ones (2a-h) and (3a-h), azetidin-2-one (4a-h) and 1,3,4-oxadiazole derivatives (5a-h), (E)-N-(2-substituted benzylidene) isonicotinohydrazides (1a-h) were prepared starting from isonicotinic acid hydrazide. The reaction of isonicotinic acid hydrazide with substituted benzaldehyde in refluxing methanol with few drops of glacial acetic acid gave the (E)-N-(2-substituted benzylidene)isonicotinohydrazides (1a-h). In the present study, the reaction of the substituted benzylidene isonicotinohydrazides (1a-h) with thioglycolic acid, thiomalic acid, (4H, m, aromatic), 2.73 (6H, s, N(CH3)2). 13C-NMR (100 MHz, CDCl3, δ/ppm): 7.65, 8.33 (4H, m, Py), 7.26 (1H, s, CH-oxadiazole), 7.14-7.18 (4H, m, aromatic), 2.73 (6H, s, N(CH3)2). 13C-NMR (100 MHz, CDCl3, δ/ppm): 168.8, 157.0, 149.4, 149.1, 138.4, 129.8, 129.6, 128.3, 128.1, 124.1, 120.8, 112.1, 112.6, 83.8, 23.4. MS (m/z): 283 [M+].

Assessment of liver function

Liver functions such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were assessed by a reported method [21]. The alkaline phosphatase was also measured according to the reported procedures [22,23]. All data are recorded in Table 2.

Results and Discussion

Chemistry

The key intermediates used in the synthesis of thiazolidin-4-ones (2a-h) and (3a-h), azetidin-2-one (4a-h) and 1,3,4-oxadiazole derivatives (5a-h), (E)-N-(2-substituted benzylidene) isonicotino-
The antifungal activity study revealed that all the compounds tested showed good to moderate antifungal activity against all pathogenic strains (MIC 6.25 μg/mL, 12.5 μg/mL, 25 μg/mL and 50 μg/mL). Structure and biological activity relationship of title compounds showed that the presence of 4-chloro phenyl, 4-methoxy phenyl groups (MIC 6.25 and 12.5 μg/mL) are responsible for good antifungal activity.

Thus, various thiazolidine (2a-h, 3a-h), azetidine (4a-h) and oxadiazole (5a-h) derivatives of isoniazid were prepared with the objective of developing better antifungal agents. The derivatives of the aforementioned rings were found to have a promising class of compounds with an interesting pharmacological profile. Further, it is clear from structure activity relationship (SAR), that the thiazolidine derivatives (2b, 2e, 3b, 3e) were found to be more active than azetidine (4b, 4e) and oxadiazole (5b, 5e) derivatives.

### Assessment of liver function

The most active compounds (2b, 4b and 5b) of the series were evaluated further for their hepatotoxic effects by assessing the liver enzymes. Any significant changes in the level of enzymes are indicative of liver disorders. Levels of alkaline phosphatase, SGOT and SGPT enzymes were measured and the results are expressed as mean ± SEM. Compound 4b showed significant rise in the alkaline phosphatase and SGPT level with P<0.05 when compared to control. The rise in SGOT level was also found to be significant with P<0.05. Compound 5b was also found to increase the alkaline phosphatase and SGPT levels significantly with P<0.05. The rise in SGOT level was not significant

### Table 1: Antifungal activity of the synthesized compounds.

| Compounds | C. albicans MIC in μg/mL | M. niger MIC in μg/mL | M. flavus MIC in μg/mL | M. purpureus MIC in μg/mL | P. citrinum MIC in μg/mL |
|-----------|-------------------------|-----------------------|------------------------|--------------------------|-------------------------|
| 2a        | 25 (67)                 | 25 (67)               | 25 (67)                | 50 (51)                  |
| 2b        | 6.25 (100)              | 6.25 (91)             | 6.25 (97)              | 12.5 (86)                | 6.25 (97)              |
| 2c        | 50 (51)                 | 50 (47)               | 12.5 (80)              | 25 (67)                  |
| 2d        | 100 (34)                | 100 (34)              | 100 (34)               | 100 (30)                 |
| 2e        | 12.5 (74)               | 12.5 (77)             | 12.5 (80)              | 6.25 (86)                | 12.5 (77)              |
| 2f        | 100 (30)                | 100 (24)              | 100 (34)               | 100 (27)                 | 100 (34)               |
| 2g        | 25 (67)                 | 50 (25)               | 25 (50)                | 25 (64)                  |
| 2h        | 50 (51)                 | 50 (41)               | 12.5 (83)              | 50 (51)                  |
| 3a        | 25 (64)                 | 50 (47)               | 25 (61)                | 25 (64)                  |
| 3b        | 12.5 (77)               | 12.5 (74)             | 6.25 (86)              | 25 (74)                  |
| 3c        | 50 (44)                 | 25 (57)               | 25 (54)                | 25 (61)                  |
| 3d        | 100 (30)                | 100 (27)              | 100 (24)               | 25 (64)                  |
| 3e        | 12.5 (77)               | 6.25 (86)             | 6.25 (86)              | 12.5 (74)                | 12.5 (77)              |
| 3f        | 100 (30)                | 100 (24)              | 100 (21)               | 100 (24)                 | 100 (27)               |
| 3g        | 50 (47)                 | 25 (64)               | 25 (67)                | 25 (61)                  | 50 (51)                |
| 3h        | 25 (64)                 | 50 (44)               | 25 (67)                | 50 (47)                  | 50 (54)                |
| 3i        | 25 (57)                 | 50 (37)               | 50 (41)                | 50 (37)                  |
| 3j        | 12.5 (77)               | 12.5 (80)             | 12.5 (74)              | 25 (83)                  | 25 (74)                |
| 4a        | 25 (57)                 | 50 (41)               | 25 (54)                | 25 (54)                  | 50 (41)                |
| 4b        | 100 (15)                | 100 (12)              | 100 (18)               | 100 (24)                 | 100 (15)               |
| 4c        | 12.5 (74)               | 25 (64)               | 12.5 (74)              | 12.5 (77)                |
| 4d        | 100 (12)                | 100 (15)              | 100 (12)               | 100 (18)                 |
| 4e        | 25 (57)                 | 25 (57)               | 25 (54)                |
| 4f        | 25 (61)                 | 50 (41)               | 50 (37)                | 50 (41)                  |
| 4g        | 50 (47)                 | 25 (64)               | 25 (67)                |
| 4h        | 25 (67)                 | 12.5 (70)             | 12.5 (83)              |
| 5a        | 25 (57)                 | 50 (54)               | 25 (61)                |
| 5b        | 12.5 (70)               | 12.5 (70)             | 12.5 (70)              |
| 5c        | 50 (57)                 | 50 (54)               | 25 (61)                |
| 5d        | 100 (18)                | 100 (12)              | 100 (15)               |
| 5e        | 12.5 (70)               | 25 (67)               | 12.5 (70)              |
| 5f        | 100 (7)                 | 100 (7)               | 100 (3)                |
| 5g        | 50 (41)                 | 25 (61)               | 50 (37)                |
| 5h        | 12.5 (74)               | 25 (57)               | 25 (57)                |

Table 2: Enzyme estimation of the selected compounds.

| Treatment | Alkaline phosphatase ± SEM | SGOT ± SEM | SGPT ± SEM |
|-----------|-----------------------------|------------|------------|
| Control   | 12.45 ± 0.21                | 168.13 ± 1.06 | 20.19 ± 0.12 |
| 2b        | 14.32 ± 0.11                | 169.12 ± 1.11 | 28.10 ± 0.13 |
| 4b        | 38.35 ± 0.16**              | 187.32 ± 1.01 | 45.31 ± 0.16** |
| 5b        | 30.12 ± 0.13*               | 175.130.91 | 39.12 ± 0.12* |

*P<0.05; **P<0.01. The mean level of SGOT/SGPT ± SEM was calculated using ANOVA followed by Dunnett’s multiple comparison test.

### Scheme 1: Synthetic route for the title compounds.

1,2,3,4,5 R: [a = o-Cl, b = p-Cl, c = o-OH, d = m-OH, e = p-OCH3, f = p-F, g = o-NO2, h = p-N(CH3)2]
with compound 5b. Compound 2b showed no significant change in all the three enzymes and can be considered to have no hepatotoxicity.

Conclusion

Thus, various thiazolidin-4-ones, (2a-h, 3a-h) azetidin-2-ones (4a-h) and 1,3,4-oxadiazole (5a-h) derivatives of isonicizid were prepared with the objective of developing better antifungal agents. All the derivatives were found to have a promising class of compounds with an interesting pharmacological profile. Among these the compound N-(2-(4-Chlorophenyl)-4-oxothiazolidin-3-yl) isonicotinamide (2b) showed maximum antifungal activity with no hepatotoxicity effect. Hence, it is clear from structure activity relationship (SAR), that thiazolidin-4-ones derivatives were more active than azetidin-2-ones and 1,3,4-oxadiazole derivatives. Also a common result was obtained for parent drug isoniazid, which showed moderate activity against all pathogenic fungal strains. In conclusion, the isoniazid incorporated hydrazozone derivatives can be regarded as a newer class of antifungal agents. They were also found to be less toxic which indicates better tolerability of the compounds having strong future prospects.

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References

1. Gilani SJ, Khan SA, Alam O, Siddiqui N (2011) Synthesis and in vitro antitubercular activity evaluation of condensed heterocyclic 1,3,4-thiadiazole-2,5-diones and 1,2,4-triazole derivatives as antimicrobials. Eur J Med Chem 41: 531-538.
2. Nogradi T, Weaver FD (2005) Medicinal Chemistry: A Molecular & Biochemical Approach. Oxford University Press, pp. 559-582.
3. Swamy SN, Basappa, Priya BS, Prabhuswamy B, Doreswamy BH, et al. (2006) Synthesis of pharmacologically important condensed heterocyclic 4,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives as antimicrobials. J Natl Sci Acad 33: 208-214.
4. Mohan J, Kumar A (2003) Bridgehead nitrogen heterocyclic systems: Synthesis and antimicrobial activity of imidazo[2,1-b][1,3,4-thiadiazolo[2,3-c]-]s-thiadiazoles and s-triazolo[3,4-b]-1,3,4-thiadiazoles. Indian J Heterocycl Chem 12: 189–192.
5. Vignola MG, Ottanà R, Monforte F, Maccari R, Monforte MT, et al. (2003) Chiral 2,3-unsaturations: Synthesis, antimicrobial activity of a new class of heterocycles pyrrolyl oxadiazoles/thiadiazoles/1,2,4-triazoles and 1,3,4-thiadiazole derivatives of isoniazid. Acta Pol Pharm 68: 205-211.
6. Nogradi T, Weaver FD (2005) Medicinal Chemistry: A Molecular & Biochemical Approach. Oxford University Press, pp. 559-582.
7. Swamy SN, Basappa, Priya BS, Prabhuswamy B, Doreswamy BH, et al. (2006) Synthesis of pharmacologically important condensed heterocyclic 4,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives as antimicrobials. Eur J Med Chem 41: 531-538.
8. Mohan J, Kumar A (2003) Bridgehead nitrogen heterocyclic systems: Synthesis and antimicrobial activity of imidazo [2,1-b]-1,3,4-thiadiazolo[2,3-c]-]s-thiadiazoles and s-triazolo [3,4-b]-1,3,4-thiadiazoles. Indian J Heterocycl Chem 12: 189–192.
9. Kurup R, Balakrishnan S (2007) Synthesis and antidiabetic activity of a new class of heterocycles pyrrolyl oxadiazoles/thiadiazoles/1,2,4-triazoles and 1,3,4-thiadiazole derivatives of isoniazid. J Enzyme Inhib Med Chem 22: 277-284.
10. Arthington-Skaggs BA, Motley M, Warnock DW, Morrison CJ (2000) Comparative evaluation of PASCO and national committee for clinical laboratory standards M27-A broth microdilution methods for antifungal drug susceptibility testing of yeasts. J Clin Microbiol 38: 2254-2260.
11. Verma SR, Khan ZK, Singh AP (1998) Antifungal Agents: Past, Present and Future Prospects. National Academy of Chemistry and Biology, India, pp 55-58.
12. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28: 56-63.
13. King EJ, Armstrong AR (1934) A Convenient method for determining serum and bile phosphatase activity. Can Med Assoc J 31: 376-381.