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Synthesis, Antifungal and Toxicity Screening of Newer Isoniazid Derivatives

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Abstract

A series of Isoninicotinic 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 antidiabetic [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, anticancer 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, to identify new candidates that may be of value in designing new, potent, selective and less toxic antifungal agents, 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:ethyl acetate: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 ^{1}H-NMR spectra were recorded on a Bruker model DPX 300 FT-NMR spectrometer in $CDCl_3$ using tetramethylsilane (Me_4Si, 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 $C_{13}H_{10}N_3OCl$ (MW 259.69): C, 60.12; H, 3.88; N,16.18%. Found: C, 60.10; H, 3.86; N, 16.16%. IR (KBr, cm^{-1}): 3300 (N-H stretching), 1680 (C=O stretching of carbonyl), 1600 (-N=CH-Ar stretching of aromatic ring), 830 (C-Cl stretching of chlorine). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.9 (1H, s, -N=CH),

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7.72, 8.63 (4H, m, Py), 7.12-7.15 (4H, m, J = 9 Hz, aromatic), 6.1 (1H, s, NH). MS (m/z): 259 $[M]^+$.

(E)-N'-(4-Chlorobenzylidene) isonicotinohydrazide (1b). Yield: 90%; m.p.192-194°C. Anal. Calcd. for $C_{13}H_{10}N_3OCl$ (MW 259.69): C, 60.12; H, 3.88; N,16.18%. Found: C, 60.11; H, 3.87; N, 16.17%. IR (KBr, cm^{-1}): 3310 (N-H stretching), 1676 (C=O stretching of carbonyl), 1615 (-N=CH-Ar stretching of aromatic ring), 822 (C-Cl stretching of chlorine). ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 7.72, 8.63 (4H, *m*, Py), 7.12-7.15 (4H, *m, J* = 9 Hz, aromatic,), 7.7 (1H, bs, -N=CH), 6.4 (1H, *s*, NH). MS (*m/z*): 259 $[M]^+$.

(E)-N'-(2-Hydroxybenzylidene)isonicotinohydrazide (1c): Yield: 80%; m.p.168-170°C. Anal. Calcd. for $C_{13}H_{11}N_3O_2$ (MW 241.25): C, 64.72; H, 4.60; N,17.42%. Found: C, 64.70; H, 4.59; N, 17.40%. IR (KBr, cm^{-1}): 3308 (N-H stretching), 1684 (C=O stretching of carbonyl), 1612 (-N=CH-Ar stretching of aromatic ring). ^{1}H-NMR (300 MHz, $CDCl_3$, *δ*/ppm): 9.40 (1H, *br*, OH), 7.8 (1H, *s*, -N=CH), 7.13-7.16 (4H, *m, J* = 9 Hz, aromatic), 7.70, 8.61 (4H, *m,* Py), 6.2 (1H, *s*, NH). MS (*m/z*): 241$[M]^+$.

(E)-N'-(3-Hydroxybenzylidene)isonicotinohydrazide (1d): Yield: 75%; m.p.186-188°C. Anal. Calcd. for $C_{13}H_{11}N_3O_2$ (MW 241.25): C, 64.72; H, 4.60; N,17.42%. Found: C, 64.70; H, 4.59; N, 17.40%. IR (KBr, cm^{-1}): 3318 (N-H stretching), 1688 (C=O stretching of carbonyl), 1607 (-N=CH-Ar stretching of aromatic ring). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.40 (1H, br, OH), 7.7 (1H, s, -N=CH), 7.74, 8.62 (4H, m, Py), 7.11-7.14 (4H, m, J = 9 Hz, aromatic), 6.3 (1H, s, NH). MS (m/z): 241 $[M]^+$.

(E)-N'-(4-Methoxybenzylidene)isonicotinohydrazide (1e): Yield: 80%; m.p.194-196°C. Anal. Calcd. for $C_{14}H_{13}N_3O_2$ (MW 255.27): C, 65.87; H, 5.13; N, 16.46%. Found: C, 65.85; H, 5.10; N, 16.44%. IR (KBr, cm^{-1}): 3314 (N-H stretching), 1687 (C=O stretching of carbonyl), 1604 (-N=CH-Ar stretching of aromatic ring). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.73, 8.64 (4H, m, Py), 7.3 (1H, s, -N=CH), 7.13-7.16 (4H, m, J = 9 Hz, aromatic), 6.4 (1H, s, NH), 3.83 (3H, s, OCH_3). MS (m/z): 255 $[M]^+$.

(E)-N'-(4-Fluorobenzylidene)isonicotinohydrazide (1f): Yield: 90%; m.p.182-184°C. Anal. Calcd. for $C_{13}H_{10}FN_3O$ (MW 243.24): C, 64.19; H, 4.14; N, 17.28%. Found: C, 64.16; H, 4.12; N, 17.26%. IR (KBr, cm^{-1}): 3305 (N-H stretching), 1684 (C=O stretching of carbonyl), 1611 (-N=CH-Ar stretching of aromatic ring). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.70-8.61 (4H, m, Py), 7.6 (1H, s, -N=CH), 7.11-7.14 (4H, m, J = 9.0 Hz, aromatic), 6.3 (1H, s, NH). MS (m/z): 243 $[M]^+$.

(E)-N'-(2-Nitrobenzylidene)isonicotinohydrazide (1g): Yield: 85%; m.p. 188-189°C. Anal. Calcd. for $C_{13}H_{10}N_4O_3$ (MW 270.24): C, 57.78; H, 3.73; N, 20.73%. Found: C, 57.76; H, 3.70; N, 20.71%. IR (KBr, cm^{-1}): 3311 (N-H stretching), 1685 (C=O stretching of carbonyl), 1610 (-N=CH- Ar stretching of aromatic rings), 1366 (NO_2). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.8 (1H, s, -N=CH), 7.73, 8.61 (4H, m, Py), 7.10-7.13 (4H, m, J = 9.0 Hz, aromatic), 6.2 (1H, s, NH). MS (m/z): 270 $[M]^+$.

E)-N'-(4-(Dimethylamino)benzylidene)isonicotinohydrazide (1h): Yield: 85%; m.p. 196-198°C. Anal. Calcd. for $C_{15}H_{16}N4O$ (MW 268.31): C, 67.15; H,6.01; N, 20.88%. Found: C, 67.12; H, 6.00; N, 20.86%. IR (KBr, cm^{-1}): 3313 (N-H stretching), 1683(C=O stretching of carbonyl), 1613 (-N=CH-Ar stretching of aromatic rings). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.9 (1H, s, -N=CH), 7.72, 8.63 (4H, m, Py), 7.12-7.15 (4H, m, J = 9.0 Hz aromatic), 6.1 (1H, s, NH), 2.63 (6H, s, $N(CH_3)_2$). MS (m/z): 268 $[M]^+$.

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 (0.01mol) was heated on an oil-bath at 120-25°C for 12h.The reaction mixture was cooled and treated with10%sodium bicarbonate solution. The product was isolated and recrystallised from methanol-dioxane (4:1) to give compounds 2a-h.

N-(2-(2-Chlorophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2a): Yield: 85%; m.p. 198-200°C. Anal. Calcd. for $C_{15}H_{12}ClN_3O_2S$ (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), 1700 (C=O thiazolidinone), 1670 (C=O stretching of carbonyl), 1610 (C=N), 1574 (C=C stretching of chlorine), 830 (C-Cl stretching of chlorine), 700 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.8 (1H, s, CONH-), 7.74-8.64 (4H, m, Py), 7.20 (1H, s, N-CH-), 7.12-7.15 (4H, m, aromatic), 5.96 (1H, s, -S-CH-), 3.50 (2H, s, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 163.7, 149.7, 140.8, 134.0, 130.1, 128.7, 128.5, 126.7, 121.7, 102.5, 59.2, 35.6. MS (m/z): 333 $[M^+]$.

N-(2-(4-Chlorophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2b): Yield: 85%; m.p. 202-204°C. Anal. Calcd. for $C_{15}H_{12}ClN_3O_2S$ (MW 333.79): C, 53.97; H, 3.62; N, 12.59%. Found: C, 53.96; H, 3.61; N, 12.58%. IR (KBr, cm^{-1}): 3310 (N-H stretching), 1706 (C=O thiazolidinone), 1666 (C=O stretching of carbonyl),1612 (C=N), 1573 (C=C), 830 (C-Cl stretching of chlorine), 700 (C-S-C).^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.8 (1H, s, CONH-), 7.74, 8.64 (4H, m, Py), 7.2 (1H, s, N-CH-), 7.12-7.15 (4H, m, aromatic), 5.96 (1H, s, -S-CH-), 3.50 (2H, s, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 163.7, 149.7, 140.8, 134.0, 130.1, 128.7, 128.5, 126.7, 121.7, 102.5, 59.2, 35.6. MS (m/z): 333 $[M^+]$.

N-(2-(2-Hydroxyphenyl)-4-oxothiazolidin-3-yl) isonicotinamide (2c): Yield: 70%; m.p. 200-202°C. Anal. Calcd. for $C_{15}H_{13}N_3O_3S$ (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), 1674 (C=O stretching of carbonyl), 1626 (C=N), 1587 (C=C), 644 (C-S-C). ^{1}H-NMR (300 MHz, CDCl3, δ/ppm): 9.41 (1H, br, OH), 9.14 (1H, s, CONH-), 7.77-8.68 (4H, m, Py), 7.20 (1H, s, N-CH-), 7.17-7.20 (4H, m, Ar-H), 5.16 (1H, s, -S-CH-), 3.50 (2H, s, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 163.7, 153.7,149.7, 140.8, 128.5, 128.0, 121.2, 121.7, 118.1,115.8, 58.1, 35.6. MS (m/z): 315 $[M^+]$.

N-(2-(3-Hydroxyphenyl)-4-oxothiazolidin-3-yl) isonicotinamide (2d): Yield: 70%; m.p. 208-210°C. Anal. Calcd. for $C_{15}H_{13}N_3O_3S$ (MW 315.07): C, 57.13; H, 4.16; N, 13.33%. Found: C, 57.10; H, 4.13; N, 13.32%. IR (KBr, cm^{-1}): 3317 (N-H stretching), 1717 (C=O thiazolidinone), 1672 (C=O stretching of carbonyl), 1624 (C=N), 1588 (C=C), 642 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.42 (1H, br, OH), 9.13 (1H, s, CONH-), 7.76-8.67 (4H, m, Py), 7.10 (1H, s, N-CH-), 7.17-7.20 (4H, m, aromatic), 5.16 (1H, s, -S-CH-), 3.50 (2H, s, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 163.7, 153.7, 149.7, 140.8, 128.5, 128.0, 121.2, 121.7, 118.1, 115.8, 58.1, 35.6. MS (m/z): 315 $[M^+]$.

N-(2-(4-Methoxyphenyl)-4-oxothiazolidin-3-yl) isonicotinamide (2e): Yield: 75%; m.p. 204-206°C. Anal. Calcd. for $C_{16}H_{15}N_3O_3S$ (MW 329.37): C, 58.34; H, 4.59; N, 12.76%. Found: C, 58.33; H, 4.58; N, 12.74%. IR (KBr, cm^{-1}): 3302 (N-H stretching), 1760 (C=O thiazolidinone), 1632 (C=O stretching of carbonyl), 1667 (C=N), 1546 (C=C), 628 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.06 (1H, s, CONH-), 7.70, 8.64 (4H, m, Py), 7.40 (1H, s, N-CH-), 7.26-7.32 (4H, m, aromatic), 5.22 (1H, s, -S-CH-), 3.84 (3H, s, OCH3), 3.80

(2H, s, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, 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: 85%; m.p. 196-200°C. Anal. Calcd. for $C_{15}H_{12}FN_3O_2S$ (MW 317.34): C, 56.77; H,3.81; N, 5.99%. Found: C, 56.76; H, 3.80; N, 5.98%. IR (KBr, cm^{-1}): 3316 (N-Hstretching), 1769 (C=O thiazolidinone), 1661 (C=N), 1649 (C=O stretching ofcarbonyl), 1535 (C=C), 623 (C-S-C). 1H-NMR (300 MHz, $CDCl_3$, δ/ppm):9.02 (1H, s, CONH-), 7.80 (1H, s, N-CH-), 7.75-8.60 (4H, m, Py), 7.30, 7.36(4H, m, aromatic), 5.28 (1H, s, -S-CH-), 3.10 (2H, s, CH2). ^{13}C-NMR (100MHz, $CDCl_3$, δ/ppm): 168.8, 163.7, 161.3, 149.7, 140.8, 134.8, 130.3, 121.7, 115.4, 64.3, 35.6. MS (m/z): 317 [M^+].

N-(2-(2-Nitrophenyl)-4-oxothiazolidin-3-yl)isonicotinamide (2g): Yield: 80%; m.p. 210-212°C. Anal. Calcd. for $C_{15}H_{12}N_4O_4S$ (MW 344.35): C, 52.32; H, 3.51; N, 16.27%. Found: C, 52.31; H, 3.50; N, 16.25%. IR (KBr, cm^{-1}): 3328 (N-H stretching), 1774 (C=O thiazolidinone), 1660 (C=N), 1652 (C=O stretching of carbonyl), 1532 (C=C), 620 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.05 (1H, s, CONH-), 7.90 (1H, s, N-CH-), 7.78, 8.64 (4H, m, Py), 7.31-7.34 (4H, m, aromatic), 5.21 (1H, s, -S-CH-), 3.11 (2H, s, CH_2). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 163.7, 149.7, 149.0, 140.8, 134.7, 133.4, 129.6, 128.0, 124.8, 121.7, 59.7, 35.6. MS (m/z): 344 [M^+].

N-(2-(4-(Dimethylamino)phenyl)-4-oxothiazolidin-3-yl) isonicotinamide (2h): Yield: 75%; m.p. 204-206°C. Anal. Calcd. for $C_{17}H_{18}N_4O_2S$ (MW 342.42): C, 59.63; H, 5.30; N, 16.36%. Found: C, 59.61; H, 5.29; N, 16.34%. IR (KBr, cm^{-1}):3336 (N-H stretching), 1777 (C=O thiazolidinone), 1661 (C=N), 1635 (C=O stretching of carbonyl), 1539 (C=C), 625 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$,δ/ppm): 9.03 (1H, s, CONH-), 7.71, 8.60 (4H, m, Py), 7.70 (1H, s, N-CH-), 7.28-7.32 (4H, m, aromatic), 5.20 (1H, s, -S-CH-), 3.09 (2H, s, CH_2), 2.65 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 163.7, 149.7, 149.5, 140.8, 128.7, 127.4, 121.7, 112.8, 64.3, 41.3, 35.6. MS (m/z): 342 [M^+].

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

A mixture of 1 (0.01mol) and thiomalic acid (0.01mol) was heated on an oil-bath at 120-125°C for 12h. The reaction mixture was cooled and treated with10%sodium bicarbonate solution. The product was isolated and recrystallised from methanol-dioxane (4:1) to give compounds 3a-h.

2-(2-(2-Chlorophenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3a): Yield: 80%; m.p. 210-212°C. Anal. Calcd. for $C_{17}H_{14}ClN_3O_4S$ (MW 391.83): C, 52.11; H, 3.60; N, 10.72%. Found: C, 52.10; H, 3.59; N, 10.70%. IR (KBr, cm^{-1}): 3200 (N-H stretching), 1700 (C=O thiazolidinone), 1666 (C=O stretching of carbonyl), 1610 (C=N), 1572 (C=C), 700 (C-S-C), 830 (C-Cl stretching of chlorine). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.00 (1H, s, COOH), 9.40 (1H, s, CONH-), 7.71-8.60 (4H, m, Py), 7.20 (1H, s, N-CH-), 6.17--6.15 (4H, m, Ar-H), 5.95 (1H, s, -S-CH-Ar). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 175.3, 173.3, 163.7, 149.7, 140.8, 134.0, 130.1, 121.7, 128.7, 128.5, 126.7, 102.5, 56.7, 47.5, 39.2. MS (m/z): 391 [M^+].

2-(2-(4-Chlorophenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3b): Yield: 85%; m.p. 216-218°C. Anal. Calcd. for $C_{17}H_{14}ClN_3O_4S$ (MW 391.83): Calcd: C, 52.11; H, 3.60; N, 10.72%. Found: C, 52.09; H, 3.58; N, 10.71%. IR (KBr, cm^{-1}): 3204 (N-H stretching), 1702 (C=O thiazolidinone), 1664 (C=O stretching of carbonyl), 1611 (C=N), 1572 (C=C), 701 (C-S-C), 832 (C-Cl stretching of chlorine). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.10 (1H, s, COOH), 9.50 (1H, s, CONH-), 7.72-8.61 (4H, m, Py), 7.10 (1H, s, N-CH-), 6.18-6.16 (4H, m, aromatic), 5.94 (1H, s, -S-CH-Ar). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 175.3, 173.3, 163.7, 149.7, 140.8, 134.0, 130.1, 121.7, 128.7, 128.5, 126.7, 102.5, 56.7, 47.5, 39.2. MS (m/z): 391 [M^+].

2-(2-(2-Hydroxyphenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3c): Yield: 75%; m.p. 222-224°C. Anal. Calcd. for $C_{17}H_{15}N_3O_5S$ (MW 373.38): C, 54.68; H, 4.05; N, 11.25%. Found: C, 54.67; H, 4.03; N, 10.23%. IR (KBr, cm^{-1}): 3324 (N-H stretching), 1714 (C=O thiazolidinone), 1660 (C=O stretching of carbonyl), 1625 (C=N), 1579 (C=C), 710 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.02 (1H, s, COOH), 9.40 (1H, s, CONH-), 9.33 (1H, br, OH), 7.69-8.51 (4H, m, Py), 7.50 (1H, s, N-CH-), 6.20-6.17 (4H, m, aromatic), 5.90 (1H, s, -S-CH-Ar). ^{13}C-NMR (100 MHz, CDCl3, δ/ppm): 175.3, 173.3, 163.7,153.7, 149.7, 140.8, 128.5, 128.0, 121.2, 118.1, 55.6, 47.5, 39.2. MS (m/z): 373[M^+].

2-(2-(3-Hydroxyphenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3d): Yield: 60%; m.p. 238-240°C. Anal. Calcd. for $C_{17}H_{15}N_3O_5S$ (MW 373.38): C, 54.68; H, 4.05; N, 11.25%. Found: C, 54.66; H, 4.04; N, 10.24%. IR (KBr, cm^{-1}): 3325 (N-H stretching), 1713 (C=O thiazolidinone), 1662 (C=O stretching of carbonyl), 1624 (C=N), 1580 (C=C), 711 (C-S-C). ^{1}H-NMR (300MHz, $CDCl_3$, δ/ppm): 10.02 (1H, s, COOH), 9.40 (1H, s, CONH-), 9.32 (1H,br, OH), 7.68-8.52 (4H, m, Py), 7.50 (1H, s, N-CH-), 6.20-6.17 (4H, m, aromatic), 5.90 (1H, s, -S-CH-Ar). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 175.3, 173.3, 163.7, 153.7, 149.7, 140.8, 128.5, 128.0, 121.2, 118.1, 55.6, 47.5, 39.2. MS (m/z): 373 [M^+].

2-(3-Isonicotinamido-2-(4-methoxyphenyl)-4-oxothiazolidin-5-yl)acetic acid (3e): Yield: 65%; m.p. 220-222°C. Anal. Calcd. for $C_{18}H_{17}N_3O_5S$ (MW 387.41): C, 55.80; H, 4.42; N, 10.85%. Found: C, 55.78; H, 4.41; N, 10.83%. IR (KBr, cm^{-1}): 3330 (N-H stretching), 1720 (C=O thiazolidinone), 1669 (C=O stretching of carbonyl), 1629 (C=N), 1574 (C=C), 714 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.01 (1H, s, COOH), 9.60 (1H, s, CONH-), 7.70 (1H, s, N-CH-), 7.63, 8.55 (4H, m, Py), 6.22-6.18 (4H, m, Ar-H), 5.91 (1H, s, -S-CH-Ar), 3.80 (3H, s, $-OCH_3$). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 175.3, 173.3, 163.7, 159.0, 149.7, 140.8, 131.5, 129.7, 121.7, 114.2, 61.8, 55.8, 47.5, 39.2. MS (m/z): 387 [M^+].

2-(2-(4-Fluorophenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)acetic acid (3f): Yield: 75%; m.p. 190-192°C. Anal. Calcd. for $C_{17}H_{14}FN_3O_4S$ (MW 375.37): C, 54.39; H, 3.76; N, 11.19%. Found: C, 54.38; H, 3.74; N, 11.18%. IR (KBr, cm^{-1}): 3316 (N-H stretching), 1714 (C=O thiazolidinone), 1670 (C=O stretching of carbonyl), 1634 (C=N), 1578 (C=C), 720 (C-S-C). 1H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.04 (1H, s, COOH), 9.20 (1H, s, CONH-), 7.90 (1H, s, N-CH-),7.66-8.58 (4H, m, Py), 6.20-6.17 (4H, m, aromatic), 5.93 (1H, s, -S-CH-Ar). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 175.3, 173.3, 163.7, 161.3, 149.7, 140.8, 134.8, 130.3, 121.7, 115.4, 61.8, 47.5, 39.2. MS (m/z): 375 [M^+].

2-(3-Isonicotinamido-2-(2-nitrophenyl)-4-oxothiazolidin-5-yl)acetic acid (3g): Yield: 80%; m.p. 232-234 °C. Anal. Calcd. for $C_{17}H_{14}N_4O_6S$ (MW 402.38): C, 50.74; H, 3.51; N, 13.92%. Found: C, 50.73; H, 3.50; N, 13.91%. IR (KBr, cm^{-1}): 3310 (N-H stretching), 1704 (C=O thiazolidinone), 1660 (C=O stretching of carbonyl), 1632 (C=N), 1580 (C=C), 727 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.03 (1H, s, COOH), 9.40 (1H, s, CONH-), 7.63, 8.31 (4H, m, Py), 7.50 (1H, s, N-CH), 6.24-6.20 (4H, m, Ar-H), 5.90 (1H, s, -S-CH-Ar). ^{13}C-NMR (100 MHz, $CDCl_3$, 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-(4-(Dimethylamino)phenyl)-3-isonicotinamido-4-oxothiazolidin-5-yl)-acetic acid (3h): Yield: 70%; m.p. 248-250°C. Anal. Calcd. for $C_{19}H_{20}N_4O_4S$ (MW 400.45): C, 56.99; H, 5.03; N, 13.99%. Found: C, 56.97; H, 5.01; N, 13.98%. IR (KBr, cm^{-1}): 3315 (N-H stretching), 1710 (C=O thiazolidinone), 1666 (C=O stretching of carbonyl), 1632 (C=N), 1584 (C=C), 727 (C-S-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 10.06 (1H, s, COOH), 9.10 (1H, s, CONH-), 7.68-8.33 (4H, m, Py), 6.22-6.19 (4H, m, Ar-H), 7.10 (1H, s, N-CH-), 5.93 (1H, s, -S-CH-Ar), 2.72 (6H, s, $N(CH_3)_2$). ^{13}C-NMR (100 MHz, CDCl3, δ/ppm): 175.3, 173.3, 163.7, 149.7, 149.5, 140.8, 128.7, 127.4, 121.7, 112.8, 57.2, 47.5, 39.2. MS (m/z): 400 [M^+].

General procedure for the synthesis of N-(3-chloro-2-(2-substitutedphenyl)-4-oxaazetidin-1-yl) isonicotinamide (4a-h)

A solution of 1 (0.01 mol) in dioxane (20 mL) was added to a well stirred mixture of chloroacetylchloride (0.012 mol) and triethylamine (Et_3N) (0.012 mol) in dioxane (10 mL) at 0-5°C. The reaction mixture was then stirred for 8h, kept for 2days at room temperature and then treated with cold water. The solid thus obtained was filtered, washed with water and recrystallized from methanol to yield 4a-h.

N-(3-Chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl) isonicotinamide (4a): Yield: 75%; m.p. 322-324°C. Anal. Calcd. for $C_{15}H_{11}C_{l2}N_3O_2$ (MW 336.17): C, 53.59; H, 3.30; N, 12.50%. Found: C, 53.58; H, 3.29; N, 12.48%. IR (KBr, cm^{-1}): 3250 (N-H stretching), 1745 (C=O β-lactam ring), 1616 (C=O stretching of carbonyl), 1600 (C=N), 1560 (C=C), 742 (C-Cl stretching of chlorine). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.40 (1H, s, CONH-), 7.70 (1H, s, N-CH-), 7.68-8.33 (4H, m, Py), 6.61-6.63 (4H, m, aromatic). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 163.7, 163.5, 149.7, 143.5, 140.8, 132.2, 128.6, 128.1, 126.6, 121.7. MS (m/z): 335 [M^+].

N-(3-Chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl) isonicotinamide (4b): Yield: 65%; m.p. 328-330°C. Anal. Calcd. for $C_{15}H_{11}Cl_2N_3O_2$ (MW 336.17): C, 53.59; H, 3.30; N, 12.50%. Found: C, 53.57; H, 3.27; N, 12.46%. IR (KBr, cm^{-1}): 3252 (N-H stretching), 1746 (C=O β-lactam ring), 1612 (C=O stretching of carbonyl), 1599 (C=N), 1562 (C=C), 741 (C-Cl stretching of chlorine). ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 9.80 (1H, s, CONH-), 7.60 (1H, s, N-CH-), 7.67-8.32 (4H, m, Py), 6.62-6.64 (4H, m, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 163.7, 163.5, 149.7, 143.5, 140.8, 132.2, 128.6, 128.1, 126.6, 121.7. MS (m/z): 335 [M^+].

N-(3-Chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl) isonicotinamide (4c): Yield: 70%; m.p. 346-348°C. Anal. Calcd. for $C_{15}H_{12}ClN_3O_3$ (MW 317.73): C, 56.70; H, 3.81; N, 13.23%. Found: C, 56.68; H, 3.80; N, 13.21%. IR (KBr, cm^{-1}): 3256 (N-H stretching), 1749 (C=O β-lactam ring), 1614 (C=O stretching of carbonyl), 1602 (C=N), 1561 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.20 (1H, br, OH), 9.90 (1H, s, CONH-), 7.64-8.31 (4H, m, Py), 7.40 (1H, s, N-CH-), 6.60-6.61 (4H, m, aromatic). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 163.7, 163.5, 154.0, 149.7, 140.8, 130.9, 128.1, 126.5, 121.7, 121.1, 115.7, 64.4, 61.2. MS (m/z): 317 [M^+].

N-(3-Chloro-2-(3-hydroxyphenyl)-4-oxoazetidin-1-yl) isonicotinamide (4d): Yield: 60%; m.p. 330-332°C. Anal. Calcd. for $C_{15}H_{12}ClN_3O_3$ (MW 317.73): C, 56.70; H, 3.81; N, 13.23%. Found: C, 56.69; H, 3.80; N, 13.22%. IR (KBr, cm^{-1}): 3259 (N-H stretching), 1752 (C=O β-lactam ring), 1672 (C=O stretching of carbonyl), 1605 (C=N), 1562 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.30 (1H, br, OH), 9.80 (1H, s, CONH-), 7.64-8.31 (4H, m, Py), 7.40 (1H, s, N-CH-), 6.60-6.61 (4H, m, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$, 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. Calcd. for $C_{16}H_{14}ClN_3O_3$ (MW 331.75): C, 57.93; H, 4.25; N, 12.67%. Found: C, 57.91; H, 4.22; N, 12.65%. IR (KBr, cm^{-1}): 3260 (N-H stretching), 1748 (C=O β-lactam ring), 1670 (C=O stretching of carbonyl), 1603 (C=N), 1558 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.40 (1H, s, CONH-), 7.62, 8.31 (4H, m, Py), 7.20 (1H, s, N-CH-), 6.63-6.65 (4H, m, aromatic), 3.78 (3H, s, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 163.7, 163.5, 158.6, 149.7, 140.8, 135.8, 126.6, 121.7, 114.1, 67.4, 64.1. MS (m/z): 331 [M^+].

N-(3-Chloro-2-(4-fluorophenyl)-4-oxoazetidin-1-yl) isonicotinamide (4f): Yield: 75%; m.p. 298-300°C. Anal. Calcd. for $C_{15}H_{11}ClFN_3O_2$ (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), 1672 (C=O stretching of carbonyl), 1613 (C=N), 1560 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.60 (1H, s, CONH-), 7.60, 8.31 (4H, m, Py), 7.80 (1H, s, N-CH-), 6.62-6.67 (4H, m, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 163.7, 163.5, 160.9, 149.7, 140.8, 139.1, 128.5, 121.7, 115.3, 67.4, 64.1. MS (m/z): 319 [M^+].

N-(3-Chloro-2-(2-nitrophenyl)-4-oxoazetidin-1-yl) isonicotinamide (4g): Yield: 60%; m.p. 294-296°C. Anal. Calcd. for $C_{15}H_{11}ClN_4O_4$ (MW 346.73): C, 51.96; H, 3.20; N, 16.16%. 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), 1614 (C=N), 1562 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.40 (1H, s, CONH-), 7.63, 8.34 (4H, m, Py), 7.30 (1H, s, N-CH-), 6.64-6.66 (4H, m, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 163.7, 163.5, 149.7, 147.2, 140.8, 137.5, 134.6, 127.6, 124.7, 121.7, 63.1, 62.8. MS (m/z): 346 [M^+].

N-(3-Chloro-2-(4-(dimethylamino)phenyl)-4-oxoazetidin-1-yl) isonicotinamide (4h): Yield: 65%; m.p. 360-362°C. Anal. Calcd. for $C_{17}H_{17}ClN_4O_2$ (MW 344.80): C, 59.22; H, 4.97; N, 16.25%. Found: C, 59.21; H, 4.96; N, 16.23%. IR (KBr, cm^{-1}): 3266 (N-H stretching), 1747 (C=O β-lactam ring), 1668 (C=O stretching of carbonyl), 1611 (C=N), 1565 (C=C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.10 (1H, s, CONH-), 7.90 (1H, s, N-CH-), 7.67, 8.32 (4H, m, Py), 6.65-6.69 (4H, m, Ar-H), 2.70 (6H, s, N(CH3)2. ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 163.7, 163.5, 149.7, 149.1, 140.8, 133.0, 129.2, 121.7, 112.7, 67.4, 64.1, 41.3. MS (m/z): 344 [M^+].

General procedure for the synthesis of 1-(2-(2-substitutedphenyl)-5-(pyridine-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5a-h)

A mixture of **1**(0.003mol) and acetic anhydride (10mL) was heated under reflux for 4h. After the reaction mixture attained room temperature, excess acetic anhydride was decomposed by water and the mixture was stirred for further 30 min. The separated product was filtered, washed with water, dried and recrystallized in appropriate solvent systems to give the products **5a-h**.

1-(2-(2-Chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5a): Yield: 65%; m.p. 182-184°C. Anal. Calcd. for $C_{15}H_{12}ClN_3O_2$ (MW 301.73): C, 59.71; H, 4.01; N, 13.93%. Found: C, 59.70; H, 4.00; N, 13.91%. IR (KBr, cm^{-1}): 1660 (acetyl C=O), 1614 (C=N), 1560 (C=C), 830 (C-Cl stretching of chlorine), 1500 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.72-8.64 (4H, m, Py), 7.12-7.14 (4H, m, aromatic), 7.19 (1H, s, CH- oxadiazole). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 157.0, 149.4, 142.8, 138.4, 132.2, 128.6, 128.3, 128.1, 126.6, 124.1, 78.4, 23.4. MS (m/z): 301 [M^+].

1-(2-(4-Chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5b): Yield: 60%; m.p. 186-188°C. Anal. Calcd. for $C_{15}H_{12}ClN_3O_2$ (MW 301.73): C, 59.22; H, 4.97; N, 16.25%. Found: C, 59.21; H, 4.95; N, 16.23. IR (KBr, cm^{-1}): 1662 (acetyl C=O), 1616 (C=N), 1562 (C=C), 834 (C-Cl stretching of chlorine), 1504 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.73, 8.65 (4H, m, Py), 7.20 (1H, s, CH-oxadiazole), 7.13-7.15 (4H, m, aromatic). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 157.0, 149.4, 138.4, 132.3, 128.6, 128.3, 124.1, 83.5, 23.4. MS (m/z): 301 [M^+].

1-(2-(2-Hydroxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5c): Yield: 50%; m.p. 198-200°C. Anal. Calcd. for $C_{15}H_{13}N_3O_3$ (MW 283.28): C, 63.60; H, 4.63; N, 14.83%. Found: C, 63.58; H, 4.61; N, 14.81%. IR (KBr, cm^{-1}): 1664 (acetyl C=O), 1618 (C=N), 1563 (C=C), 1510 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.32 (1H, br, OH), 7.62, 8.34 (4H, m, Py), 7.20 (1H, s, CH-oxadiazole), 7.10-7.13 (4H, m, aromatic). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 157.0, 149.4, 138.4, 129.6, 128.3, 128.1, 124.1, 121.1, 115.7, 77.3, 23.4. MS (m/z): 283 [M^+].

1-(2-(3-Hydroxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5d): Yield: 65%; m.p. 210-212°C. Anal. Calcd. for $C_{15}H_{13}N_3O_3$ (MW 283.28): C, 63.60; H, 4.63; N, 14.83%. Found: C, 63.59; H, 4.62; N, 14.82. IR (KBr, cm^{-1}): 1664 (acetyl C=O), 1618 (C=N), 1563 (C=C), 1510 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 9.32 (1H, br, OH), 7.62, 8.34 (4H, m, Py), 7.20 (1H, s, CH-oxadiazole), 7.10-7.13 (4H, m, aromatic). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 157.0, 156.8, 149.4, 141.7, 138.4, 129.9, 124.1, 113.9, 112.6, 83.8, 23.4. MS (m/z): 283 [M^+].

1-(2-(4-Methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5e): Yield: 55%; m.p. 202-204°C. Anal. Calcd. for $C_{16}H_{15}N_3O_3$ (MW 297.31): C, 64.64; H, 5.09; N, 14.13%. Found: C, 64.62; H, 5.07; N, 14.11%. IR (KBr, cm^{-1}): 1667 (acetyl C=O), 1615 (C=N), 1561 (C=C), 1515 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.66, 8.38 (4H, m, Py), 7.22 (1H, s, CH-oxadiazole), 7.14-7.17 (4H, m, aromatic), 3.71 (3H, s, OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 157.0, 156.5, 149.4, 138.4, 127.9, 127.7, 124.1, 120.8, 112.1, 77.6, 23.4. MS (m/z): 297 [M^+].

1-(2-(4-Fluorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5f): Yield: 65%; m.p. 206-208°C. Anal. Calcd. for $C_{15}H_{12}FN_3O_2$ (MW 285.27): C, 63.15; H, 4.24; N, 14.73%. Found: C, 63.14; H, 4.23; N, 14.71%. IR (KBr, cm^{-1}): 1663 (acetyl C=O), 1620 (C=N), 1565 (C=C), 1518 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.69, 8.37 (4H, m, Py), 7.25 (1H, s, CH-oxadiazole), 7.13-7.18 (4H, m, aromatic). ^{13}C-NMR (100 MHz, CDCl3, δ/ppm): 168.8, 157.0, 159.4, 149.4, 138.4, 129.4, 128.5, 128.3, 124.1, 76.7, 23.4. MS(m/z): 285 [M^+].

1-(2-(2-Nitrophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5g): Yield: 70%; m.p. 196-198°C. Anal. Calcd. for $C_{15}H_{12}N_4O_4$ (MW 312.28): C, 57.69; H, 3.87; N, 17.94%. Found: C, 57.67; H, 3.85; N, 17.93%. IR (KBr, cm^{-1}): 1669 (acetyl C=O), 1619 (C=N), 1563 (C=C), 1517 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, δ/ppm): 7.67, 8.35 (4H, m, Py), 7.23 (1H, s, CH-oxadiazole), 7.12-7.15 (4H, m, aromatic). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 157.0, 149.4, 148.2, 139.4, 138.4, 130.9, 129.4, 127.6, 124.7, 78.9, 23.4. MS (m/z): 312 [M^+].

1-(2-(4-(Dimethylamino)phenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)-ethanone(5h): Yield: 75%; m.p. 204-206°C. Anal. Calcd. for $C_{17}H_{18}N_4O_2$ (MW 310.35): C, 65.79; H, 5.85; N, 18.05%. Found: C, 65.78; H, 5.83; N, 18.03%. IR (KBr, cm^{-1}): 1664 (acetyl C=O), 1624 (C=N), 1570 (C=C), 1511 (C-O-C). ^{1}H-NMR (300 MHz, $CDCl_3$, 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, $N(CH_3)_2$). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): 168.8, 157.0, 149.4, 149.1, 138.4, 129.8, 127.8, 124.1, 83.5, 41.3, 23.4. MS (m/z): 310 [M^+].

Biological Activity

Antifungal activity

Antifungal activity of the synthesized compounds were determined *in vitro* by using serial plate dilution method [19, 20] against *C. albicans* (ATCC 2091), *A. niger* (MTCC 281), *A. flavus* (MTCC 277), *M. purpureous* (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, chloroacetyl chloride and acetic anhydride in presence of various reagents gave the new thiazolidin-4-ones (2a-h) and (3a-h), azetidin-2-one (4a-h) and 1,3,4-oxadiazole derivatives (5a-h), respectively. The synthesis routes to the compounds are outlined in Scheme 1.

Antifungal activity

The newly synthesized compounds 2a-h, 3a-h, 4a-h and 5a-h were screened for their antifungal activity. The results of antifungal effect

Compounds	MIC in µg/mL and zone of inhibition (%)				
	C. albicans	*A. niger*	*A. flavus*	*M. purpureous*	*P. citrinum*
2a	25 (67)	50 (51)	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 (51)	50 (47)	12.5(80)	25 (67)
2d	100 (34)	100 (30)	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 (51)	25 (67)	50 (51)	25 (64)
2h	50 (51)	50 (41)	50 (47)	12.5 (83)	50 (51)
3a	25 (64)	50 (47)	25 (61)	25 (64)	50 (51)
3b	12.5 (77)	12.5 (74)	6.25 (86)	6.25 (86)	12.5 (74)
3c	50 (44)	25 (57)	25 (54)	25 (61)	50 (51)
3d	100 (30)	100 (27)	100 (24)	100 (27)	100 (34)
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)
4a	25 (57)	50 (37)	50 (41)	50 (37)	12.5 (74)
4b	12.5 (77)	12.5 (80)	12.5 (74)	25 (83)	25 (74)
4c	25 (57)	50 (41)	25 (54)	25 (54)	50 (41)
4d	100 (15)	100 (12)	100 (18)	100 (24)	100 (15)
4e	12.5 (74)	25 (64)	12.5 (74)	12.5 (77)	25 (64)6
4f	100 (12)	100 (15)	100 (12)	100 (18)	100 (18)
4g	25 (57)	25 (57)	25 (54)	25 (57)	50 (44)
4h	25 (61)	50 (41)	50 (37)	50 (41)	12.5 (77)
5a	12.5 (70)	25 (54)	25 (57)	25 (61)	50 (44)
5b	25 (67)	12.5 (70)	12.5 (83)	25 (67)	12.5 (70)
5c	25 (57)	50 (54)	25 (61)	25 (54)	12.5 (74)
5d	100 (18)	100 (12)	100 (15)	100 (18)	100 (12)
5e	12.5 (70)	25 (67)	12.5 (70)	12.5 (77)	25 (57)
5f	100 (7)	100 (7)	100 (3)	100 (12)	100 (7)
5g	50 (41)	25 (61)	50 (37)	25 (61)	25 (64)
5h	12.5 (74)	25 (57)	25 (57)	25 (54)	12.5 (77)
Ketoconazole	6.25 (100)	6.25 (100)	6.25 (100)	6.25 (100)	6.25 (100)
Isoniazid	25 (67)	25 (61)	25 (67)	50 (51)	25 (64)

Table 1: Antifungal activity of the synthesized 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*	175.13*0.91	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.

Table 2: Enzyme estimation of the selected compounds.

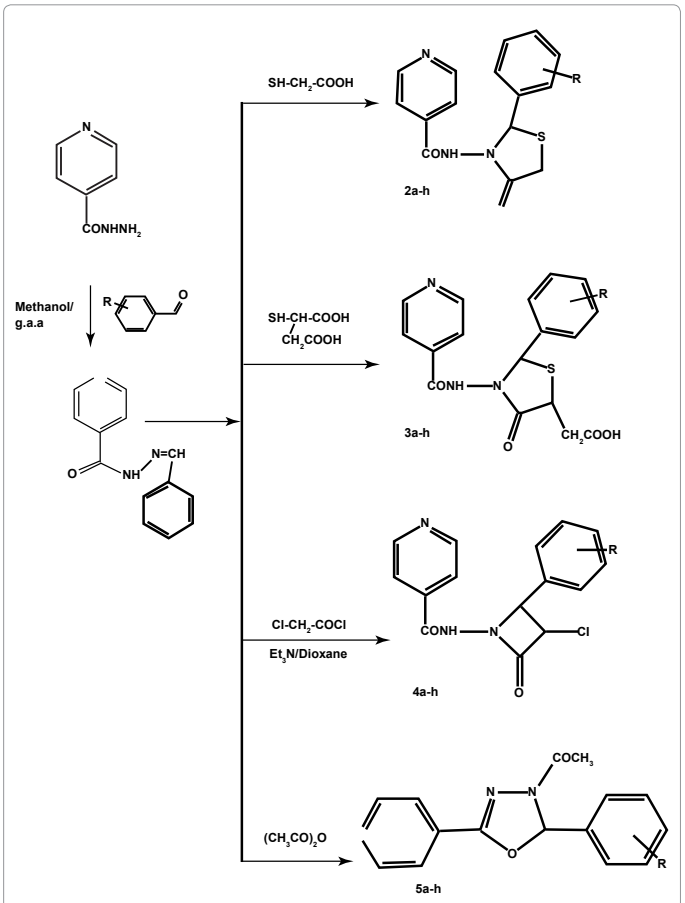
of all the tested compounds were reported as minimal inhibitory concentrations (MICs, µg/mL). The compounds 2b, 2e, 3b, 3e, 4b, 4e, 5b and 5e showed comparatively good activity against all the fungal strains (Table 1). The good activity is attributed to the presence of pharmacologically active chloro (2b, 3b, 4b, 5b) and methoxy (2e, 3e, 4e, 5e) groups attached to phenyl group at fourth position respectively (MIC 6.25 µg/mL, 12.5 µg/mL & 25 µg/mL). When these groups were replaced by *m*-hydroxy and *p*-fluoro groups (2d, 2f, 3d, 3f, 4d, 4f,5d, 5f) it caused sharp decrease in activity against most of the strains (MIC 100 µg/mL). Rest of the compounds exhibited moderate activity compared to that of standard against all the fungal strains (MIC 25µg/mL & 50 µg/mL).

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 and12.5 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<0.01 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



1,2,3,4,5 **R** : [**a** = *o*-Cl, **b** = *p*-Cl, **c** = *o*-OH, **d** = *m*-OH, **e** = *p*-OCH_3, **f** = *p*-F, **g** = *o*-NO_2, **h** = *p*-$N(CH_3)_2$]

Scheme 1: Synthetic route for the title compounds.

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 isoniazid 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 hydrazone 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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