Synthesis and Antimicrobial Activity of Phthalimido(2-Aryl-3-Isonicotinamido-4-Oxo-1,3-Thiazolidine-5-yl) Ethanoates

RANJANA SHARMA, M. AHMED, KANIKA SHARMA AND G. L. TALESARA*
Department of Botany and Department of Chemistry, M. L. Sukhadia University, Udaipur-313 001

2-Aryl-3-isonicotinamido-4-oxo-1,3-thiazolidine-5-yl ethanoic acid (2a-h) have been synthesized via cycloaddition of 4-arylidenehydrazido pyridine (1a-h) with mercaptosuccinic acid in THF containing a pinch of ZnCl₂, which on treatment with thionyl chloride give corresponding ethanoyl chloride derivatives (3a-h). These on treatment with N-hydroxyphthalimide afford titled compounds (4a-h). IR, ¹H NMR, Mass and ¹³C NMR determined their structures. All the synthesized compounds were screened for antibacterial (Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa and Salmonella typhi) and antifungal (Candida albicans and Aspergillus fumigatus) activities. All the compounds exhibited significant activity against the bacteria and fungi tested.

In the present investigation an attempt has been made to synthesize phthalimido (2-aryl-3-isonicotinamido-1,3-thiazolidine-5-yl) ethanoates and to evaluate their antimicrobial activity. Introduction of phthalimidoxy group into heterocyclic ring system generates compounds of biological interest. Several compounds such as methyl N-phthalimidoxy-2-methacrylate, ethyl N-phthalimidoxy acetate and 3-N-(4-cyanobenzyl)phthalimidoxy containing phthalimidoxy group have been demonstrated to possess anticonvulsant, anticancer, antimalarial, hypotensive, antifungal, antiamoebic and human leukocyte inhibitory properties. Isoniazid (INH) on their own exhibit moderate antimicrobial activity but their derivatives are known to possess pharmaceutical values. Equipoent antimicrobial activity of some isonicotinamide derivatives such as 2-ketoindol-3-isonicotinoyl hydrazone and 2-aryl sulphonamido-α-carbamaylaryl methylamino-5-(4'-pyridyl)-1,3,4-oxadiazole has been reported. Review of literature shows that thiazolediones are endowed a variety of biological activities. Therefore, it was planned to introduce phthalimidoxy group to thiazolidino derivatives of isonicotamide at appropriate position under reaction conditions with the object of studying their biological effects.

MATERIALS AND METHODS

Melting points of synthesized compounds were determined in open capillary tubes are therefore uncorrected. The structures of compounds were established on the basis of elemental analysis and spectral data. The IR spectra were recorded in the range of 4000-450 cm⁻¹ using KBr disc on a FTIR RXI Perkin-Elmer spectrophotometer. ¹H NMR and ¹³C NMR were recorded on a Bruker DRX 300 MHz spectrophotometer using CDCl₃/DMSO-d₆ as solvent with TMS as an internal standard. The FAB mass spectra were recorded on a Jeol SX-102/DA-6000 spectrophotometer data system using argon/xenon (6 KV, 10 mA) as FAB gas. Purity of synthesized compounds was checked by silica gel-G plate of 2 mm thickness using benzene and ethyl acetate as developer. N-Hydroxyphthalimide was prepared by the reported method.

4-Arylidenehydrazido pyridine (1a-h):

A mixture of isoniazid (13.7 g, 0.1 mol), corresponding aldehydes (0.1 mol) and glacial acetic acid (4-5 drops) in ethanol (30 ml) was heated under reflux for 4 h. The solid thus separated on cooling was filtered, washed with water, dried and recrystallized from ethanol.

2-Aryl-3-isonicotinamido-4-oxo-1,3-thiazolidine-5-yl
ethanoic acid (2a-h):

A mixture of (1a, 0.1 mol) and mercaptoacetic acid in THF containing a pinch of anhydrous ZnCl₂ was refluxed on water bath for 8 h. The separated solid was filtered, dried and recrystallized from ethanol. Compounds (2a-h) have been synthesized by similar method using reagents in proper mole ratio.

2-Aryl-3-isonicotinamido-4-oxo-1,3-thiazolidine-5-yl ethanoyl chloride (3a-h):

A solution of (2a, 0.01 mol) in benzene (25 ml) and thionyl chloride (0.02 mol) was refluxed for 60 min on water bath. Excess of thionyl chloride was removed under reduced pressure. On cooling, solid obtained, was filtered, dried and recrystallized from ethanol. Likewise ethanoyl chloride derivatives (3b-h) were also prepared.

Phthalimido(2-aryl-3-isonicotinamido-4-oxo-1,3-thiazolidine-5-yl)ethanoates (4a-h) (Scheme 1):

To a solution of compound (3a, 0.01 mol) in dry DMF (25 ml) N-hydroxyphthalimide (1.63 g, 0.01 mol) and TEA (0.01 mol) was added. The reaction mixture was stirred at room temperature for an hour. It was refluxed for 3 h. It was filtered and solvent was removed under reduced pressure. Thus, solid was obtained, dried and recrystallized from ethanol. Compounds (4b-h) were prepared by the similar process with minor modification in mole ratio of reagents by changing in refluxing time.

4a: IR (KBr, νmax in cm⁻¹): 3360 (N-H str.), 1705 (C=O, CONH), 1669 (CO-N-CO), 1507 (C=NC), 1170 (C-O), 697 (C-S); PMR (DMSO-d₆, δ in ppm): 8.7 (s, 1H, CONH), 8.4 (d, 2H, Ar-H of pyridine ring, near N), 7.6 (m, 4H, Ar-H), 7.0 (d, 2H, Ar-H), 6.9 (d, 2H, Ar-H of pyridine ring), 6.8 (d, 2H, Ar-H, near OCH₃), 3.7 (s, 3H, OCH₃), 3.3 (s, 1H, N-CH₃), 3.0 (t, 1H, CH-CH₂CO), 2.9 (d, 2H, CH-CH₂CO) ; m/z: 532 [M⁺]**, 504, 425, 204, 190, 162, 146, 132, 121, 106, 104, 76, 76.

4b: IR (KBr, νmax in cm⁻¹): 3355 (N-H str.), 1710 (C=O, CONH), 1670 (CO-N-CO), 1570 (C=NC), 1250 (C-O), 754 (C-Cl), 670 (C-S); PMR (DMSO-d₆, δ in ppm): 8.6 (s, 1H, CONH), 8.5 (d, 2H, Ar-H of pyridine ring), 7.5 (m, 4H, Ar-H), 7.49 (d, 2H, Ar-H, near Cl), 6.84 (d, 2H, Ar-H of pyridine ring), 6.8 (d, 2H, Ar-H), 3.5 (s, 1H, N-CH₃), 3.3 (t, 1H, CH-CH₂CO), 2.85 (d, 2H, CH-CH₂CO) ; m/z: 536 [M+2]**, 534 [M]**, 425, 204, 190.

4c: IR (KBr, νmax in cm⁻¹): 1717 (C=O, CONH), 1660 (CO-N-CO), 679 (C-S); PMR (DMSO-d₆, δ in ppm): 8.5 (s, 1H, CONH), 8.33 (d, 2H, Ar-H of pyridine ring), 7.2 (m, 4H, Ar-

Scheme 1: Synthetic scheme for phthalimido (2-aryl-3-isonicotinamido-4-oxo-1,3-thiazolidine-5-yl)ethanoates.

H), 6.8 (d, 2H, Ar-H of pyridine ring), 3.62 (s, 9H, OCH₃), 3.55 (s, 1H, N-CH₃), 3.43 (t, 1H, CH-CH₂CO); m/z: 592 [M⁺]**, 425, 204, 190, 162.

4d: IR (KBr, νmax in cm⁻¹): 3333 (N-H str.), 1525, 1317 (NO₂), 675 (C-S); PMR (DMSO-d₆, δ in ppm): 8.72 (s, 1H, CONH), 8.4 (d, 2H, Ar-H of pyridine ring), 8.2 (d, 2H, Ar-H, near NO₂), 8.1 (m, 4H, Ar-H), 7.6 (d, 2H, Ar-H), 6.95 (d, 2H, Ar-H of pyridine ring), 3.41 (s, 1H, N-CH₂), 3.5 (t, 1H, CH-CH₂CO), 3.1 (d, 2H, CH-CH₂CO); m/z: 547 [M⁺]**, 425, 204, 190, 162.

4f: IR (KBr, νmax in cm⁻¹): 3354 (N-H str.), 1253 (C-N), 670 (C-S); PMR (DMSO-d₆, δ in ppm): 8.6 (s, 1H, CONH), 8.49 (d, 2H, Ar-H of pyridine ring), 7.3 (m, 4H, Ar-H), 6.8 (d, 2H, Ar-H, near N(CH₃)₂), 6.7 (d, 2H, Ar-H of pyridine ring), 6.69 (d, 2H, Ar-H), 3.6 (s, 1H, N-CH₃), 3.0 (d, 2H, CH₂CO); m/z: 545 [M⁺]**, 517, 425, 204, 190, 162.

4g: IR (KBr, νmax in cm⁻¹): 3332 (N-H str.), 1675 (CO-N-CO), 1620 (C=N), 1250 (C-N), 682 (C-S); PMR (DMSO-d₆, δ in ppm): 8.62 (s, 1H, CONH), 8.46 (d, 2H, Ar-H of pyridine
ring), 7.6 (m, 9H, Ar-H), 6.92 (d, 2H, Ar-H of pyridine ring), 3.5 (s, 1H, N-CH), 3.39 (d, 2H, CH-CH₂CO); m/z: 502 [M]+, 474, 425, 204, 190, 162, 146, 132, 121.

4h: IR (KBr, ν max in cm⁻¹): 3350 (N-H str.), 3022 (Ar-H), 1712 (C=O, CONH), 1672 (CO-N=CO), 685 (C-S); PMR (DMSO-d₆, δ in ppm): 8.9 (s, 1H, CONH), 8.32 (d, 2H, Ar-H of pyridine ring), 7.8 (d, 1H, Ar-H furyl ring), 7.5 (m, 4H, Ar-H), 7.2 (d, 1H, Ar-H furyl ring), 6.93 (d, 2H, Ar-H of pyridine ring) 6.5 (quarted, 1H, Ar-H of furyl ring), 3.31 (t, 1H, CH₂CO); m/z: 492 [M]+, 425, 204, 190, 162.

Antimicrobial activity:

Antimicrobial activity was assayed by well or cup method in Nutrient agar and Sabouraud dextrose agar. Media was inoculated with 0.2 ml suspension of organisms by spread plate method. With the help of sterile borer, a well was made in the center of the medium and filled with 100 µg/ml concentration of synthesized compounds. The incubation time was 24 h at 37°C for bacteria and 74 h at 37°C for fungal strains. Antimicrobial activity was measured as a function of diameter of zone of inhibition (mm). The experiment was repeated 3 times. The results were compared to ciprofloxacin and gentamicin for antibacterial activity and griseofulvin and fluconazole for antifungal activity by measuring zone of inhibition in mm at 100 µg/ml concentration using disc diffusion method.

RESULTS AND DISCUSSION

Isoniazid on reaction with various aldehydes in ethanol produces 4-arylidenecyrazido pyridine (1a-h) in good yield. The structures of compounds (1a-h) have been proved by IR, ¹H NMR and Mass spectral data. The IR absorption due to ν (C=O) str. and ν (C=N) str. appeared at 1710-1678 cm⁻¹ and 1590-1492 cm⁻¹, respectively. The ¹H NMR spectra of these compounds in DMSO-d₆ exhibited the following signals: (δ in ppm) 9.0-8.7 (s, 1H, CONH) 8.6-8.4 (d, 2H, Ar-H, protons of pyridine ring near N) and 7.13-7.0 (s, 1H, N=CH). The mass spectrum of compound (1a) showed a fairly intense molecular ion peak at m/z 255 [M]+ confirming the molecular formula, C₁₄H₁₃N₅O₅ assigned structure for this compound. These arylidene derivatives (1a-h) on cyclodehydration with mercaptocarboxylic acid furnish 2-aryl-3-isonicotinamido-4-oxo-1,3-thiazolidine-5-yl ethanoic acid (2a-h). The reaction conditions, however, were depended upon aromatic substituents in (1a-h). Reflux time for completion of reaction varied between 8-10 h. When DMF was used as solvent, the reaction was very slow. Tetrahydrofuran and benzene gave suitable product formation. The structures of products were elucidated on the basis of physical, chemical and spectral studies. In IR spectrum, the -OH and C=O stretching bands were visible at 3100-2870 br. and 1750-1710 cm⁻¹, respectively, which showed the presence of -COOH group whereas 1675-1670 cm⁻¹ and 750-710 cm⁻¹ indicated the presence of cyclic C=O and C=S=C bonds respectively. The structures of (2a-h) also based on ¹H NMR spectrum, which reveals the presence of signals of thiazoledione -CH proton at δ 3.5-3.3 (t) ppm and -CH₂ protons at δ 3.0-2.8 (d) ppm indicating the formation of compounds (2a-h). When compounds (2a-h) have been refluxed with thiouyl chloride in benzene on a water bath for 60 min to give corresponding ethanoyl chloride derivatives (3a-h). Formations of the products were again confirmed by disappearance of the IR band at 3100-2870 br. and appearance of new IR band at 775-760 cm⁻¹ due to formation of C=Cl bond. Here the chlorine atoms of -CH₂CO-Cl are replaced by N-hydroxyphthalimide to furnish final products (4a-h). Spectral evidence such as IR, ¹H NMR, ¹³C NMR and MS spectra confirmed the structure of products (4a-h). The ¹H NMR signals at δ 8.7-8.5 (singlet), at 7.8-7.3 (multiplet) and 3.9-3.1 (doublet) confirm the presence of -CONH, Ar-H and -CH₂COO groups, respectively. Furthermore stretching of CO-N-CO around at 1680-1620 cm⁻¹ confirming the presence of imidoyl moiety in the structures of (4a-h). The absorption bands associated with other functional groups appeared in expected regions. The ¹³C NMR spectra of compound (4a) also showed the following characteristic signals: (δ in ppm) 161.4, 161.2, 150.1, 149.3 (C=O), 141.4 (2Carbon, near C=O of phthalimido ring), 129.1 (C=O), 128 (CH, near OCH₂), 128 (CH of pyridine ring, near N), 124 (CH of pyridine ring), 122 (CH of aryl ring), 114 (2CH of aryl ring), 55.4 (CH-Ar), 40.3 (CH₂CO) and 39.8 (CH₂). The structures of (4a-h) were also established from the mass spectra, which gave correct molecular ion peaks. Melting points, yields and elemental analysis of those compounds are given in Table 1.

Using 100 µg/ml concentration in DMF, the synthesized compounds (4a-h) were tested in vitro for antibacterial and antifungal activities against Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi, Candida albicans and Aspergillus fumigatus (Tables 2 and 3). The results showed that activity depends upon type and position of the substituents. For example compounds (4e) (NO₂ groups at para position) was more active than the compound (4b) (Cl-group at para position). Among phthalimido(2-aryl-3-isonicotinamido-4-oxo-1,3-thiazolidine-5-yl)ethanoates
The thiazolidino derivatives of isoniazide are substituted with phthalimidoxy group the antimicrobial activity is altered to appreciable extent. It is also observed that for antimicrobial activity, the aryl ring in compounds should be substituted with NO₂ group at para position.

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### TABLE 2: ANTIBACTERIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

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<th>Dose (µg/ml)</th>
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* Diameter of disc is 5mm.

### TABLE 3: ANTIFUNGAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

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* Diameter of disc is 5mm.

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### REFERENCES