

ANTIMICROBIAL SCREENING OF N-[(2-SUBSTITUTED PHENYL)-4-OXO-1,3-THIAZOLIDINE-3-YL]ISONICOTINAMIDES

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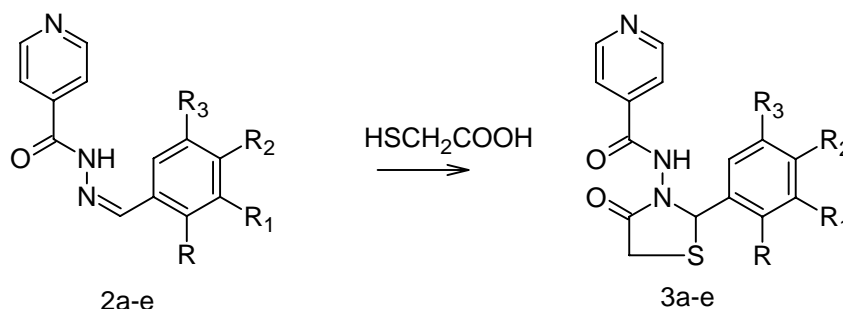
ABSTRACT

Some new N-[(2-substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]isonicotinamide derivatives (**3a-e**) have been synthesized by the reaction of N'-[(1E)-arylmethylene]isonicotinohydrazide (**2a-e**) with thioglycolic acid. These compounds were characterized on the basis of elemental and spectral analysis. The title compounds were screened for their antimicrobial activity and found to exhibit a variable degree of activity.

Key words: Antibacterial, Antifungal, Thiazolidine, MIC

INTRODUCTION

Thiazole derivatives are found to possess various biological activities viz. antibacterial¹, antifungal², antiinflammatory³, antidiabetic⁴, antihelminthic⁵, analgesic⁵, antimalarial⁶ activities. In other words the thiazole moiety is an important structural feature of many biologically active compounds. In view of such reports, we now report the synthesis of Some N-[(2-substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]isonicotinamide derivatives (**3a-e**) and antimicrobial activity associated with them. N-[(2-Substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]isonicotinamides (**3a-e**) were prepared by reacting N'-[(1E)-arylmethylene]isonicotinohydrazide (**2a-e**) with thioglycolic acid in 1,4-dioxane. The starting materials (**2a-e**) were prepared by Schiff reaction (**Scheme-1**).



SCHEME 1

EXPERIMENTAL

The melting points of the compounds were determined in open capillaries and are uncorrected. Purity of the compounds was checked by micro TLC using silica gel G coated glass plates using benzene-methanol (9:1; v/v) as irritant and iodine vapour as detecting agent. The IR (KBr) spectra were recorded on JASCO FT/IR-5300 spectrophotometer. ^1H NMR spectra ($\text{C}_6\text{D}_6/\text{CDCl}_3$) were recorded on Bruker DPX-200 MHz NMR spectrophotometer; chemical shifts (δ) are reported in ppm, with TMS as internal standard. GC Mass spectra were recorded on a Shimadzu QP 50000. Elemental analysis for C, H and N were performed on a Perkin Elmer 240 C Elemental Analyzer and were within $\pm 0.4\%$ of the theoretical values. Physical data of the compounds and percentage yield of various reactions are given in **Table-1**.

TABLE-1: PHYSICAL DATA OF N-[2-SUBSTITUTED PHENYL]-4-OXO-1,3-THIAZOLIDINE-3-YL]ISONICOTINAMIDES

Comp	R	R ₁	R ₂	R ₃	Yield (%)	m.p. ($^{\circ}\text{C}$)	m.f.	m.w.	R _f value*
3a	H	H	H	H	64	162	$\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$	300	0.56
3b	H	NO_2	H	H	58	204	$\text{C}_{15}\text{H}_{12}\text{N}_4\text{O}_4\text{S}$	345	0.62
3c	H	OCH_3	OCH_3	OCH_3	72	196	$\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$	390	0.48
3d	OH	H	H	H	60	228	$\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_3\text{S}$	316	0.64
3e	H	OCH_3	OH	H	54	212	$\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$	346	0.52

* R_f value was determined in benzene: methanol (9: 1). Recrystallization solvent – ethanol.

Synthesis of N'-(1E)-(substituted phenyl)methylene]isonicotinohydrazide (2a-e)

A mixture of isonicotinic acid hydrazide (**1**) (1.37 g; 0.01 mol) and aromatic aldehyde (0.01 mol) in 95% of ethanol were refluxed for 3-4 h. The contents were cooled and poured on to crushed ice. The crude product thus separated was filtered, dried and recrystallized from ethanol to get crystalline compound.

Synthesis of N-[2-(substituted phenyl-4-oxo-1,3-thiazolidine-3-yl)]isonicotinamide (3a-e)

To N'-(1E)-(substituted phenyl)methylene]isonicotinohydrazide (**2a-e**) (0.005 mol) in 50 ml of 1,4-dioxane, thioglycollic acid (0.92 g; 0.01 mol) was added and refluxed on a steam bath for 8 h. The contents were then cooled and poured into sodium bicarbonate solution (4N). The crude product thus separated was filtered washed with water, dried and recrystallized from ethanol.

N-[2-phenyl-4-oxo-1,3-thiazolidine-3-yl]isonicotinamide (3a): IR (KBr, cm^{-1}): 3410 (N-H), 1660 (C=O), 1570 (C=N), 1406 (CH_2CO), 1299 (C-N) and 690 (C-S); ^1H NMR (CDCl_3 , δ PPM): 8.8 (d, 1H, CO-NHN), 7.6 (d, 4H, pyridyl), 7.0 (m, 5H, 5Ar-H), 5.0 (s, 1H, CH-Ar) and 3.75 (s, 2H, S-CH₂); m/z: 300 (M^{+1}); Anal. ($\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$) Found C, 60.53%; H, 4.48%; N, 14.39%. Calculated: C, 60.18%; H, 4.38%; N, 14.04%.

N-[2-(3-nitrophenyl)-4-oxo-1,3-thiazolidine-3-yl]isonicotinamide (3b): IR (KBr, cm^{-1}): 3424 (NH), 1668 (C=O), 1580 (C=N), 1410 (CH_2-CO), 1299 (C-N) and 687 (C-S); ^1H NMR (C_6D_6 , δ PPM): 8.71 (s, 1H, CONHN), 7.7 (d, 4H, pyridyl), 7.0 (m, 4H, 4Ar-H), 5.0 (s, 1H, HC-Ar) and 3.53 (s, 2H, S-CH₂); m/z: 345 (M^{+1}); Anal. ($\text{C}_{15}\text{H}_{12}\text{N}_4\text{O}_4\text{S}$) Found C, 52.02%; H, 3.81%; N, 16.43%. Calculated: C, 52.32%; H, 3.51%; N, 16.27%.

N-[2-(3,4,5-trimethoxyphenyl)-4-oxo-1,3-thiazolidine-3-yl]isonicotinamide (3c): IR (KBr, cm^{-1}): 3423 (N-H), 1667 (C=O), 1420 (CH_2CO), 1332 (C-N) and 687 (C-S); ^1H NMR (CDCl_3 , δ PPM) δ : 8.33 (s, 1H, CONH-N), 7.7 (d, 4H, pyridyl), 7.0 (s, 2H, 2Ar-H), 5.0 (s, 1H, HC-Ar), 3.7 (s, 2H, SCH₂) and 3.2 (s, 9H, 3(OCH₃)); m/z: 390 (M^{+1}); Anal.

($C_{18}H_{19}N_3O_5S$) Found C, 55.38%; H, 5.20%; N, 10.68%. Calculated: C, 55.52%; H, 4.92%; N, 10.79%.

N-[2-(2-hydroxyphenyl)-4-oxo-1,3-thiazolidine-3-yl]isonicotinamide (3d): IR (KBr, cm^{-1}): 3424 (N-H), 1682 (C=O), 1486 (CH_2 -CO), 1274 (C-N) and 751 (C-S); 1H NMR (C_6D_6 , δ PPM): 8.71 (s, 1H, CONHN), 7.7 (d, 4H, pyridyl), 7.0 (m, 4H, 4ArH), 5.0 (s, 1H, HC-Ar) and 3.7 (s, 2H, S- CH_2); *m/z*: 316 (M^{+1}); Anal. ($C_{15}H_{13}N_3O_3S$) Found C, 57.43%; H, 4.30%; N, 13.07%. Calculated: C, 57.13%; H, 4.16%; N, 13.33%.

N-[2-(4-hydroxy-3-methoxyphenyl)-4-oxo-1,3-thiazolidine-3-yl]isonicotinamide (3e): IR (KBr, cm^{-1}): 3416 (N-H), 1655 (C=O), 1447 (CH_2 -CO), 1364 (C-N) and 683 (C-S); 1H NMR (C_6D_6 , δ PPM): 8.7 (s, 1H, CONHN), 7.7 (d, 4H, pyridyl), 7.0 (m, 3H, 3ArH), 5.0 (s, 1H, HC-Ar), 3.60 (s, 2H, S- CH_2) and 3.2 (s, 3H, OCH_3); *m/z*: 346 (M^{+1}); Anal. ($C_{16}H_{17}N_3O_4S$) Found C, 55.92%; H, 4.05%; N, 12.35%. Calculated: C, 55.64%; H, 4.38%; N, 12.17%.

Antimicrobial activity

MIC of the synthesized compounds was determined by tube dilution techniques⁷. Serial dilution of the substance under examination was placed into culture tubes containing suitable medium and inoculated with the test organism. After incubation, the minimum concentration of the test compound that inhibited the growth of organism was observed.

The synthesized compounds were evaluated for the antimicrobial activity against four bacterial strains viz. *Bacillus subtilis* (gram positive), *Staphylococcus aureus* (gram positive), *Pseudomonas aeruginosa* (gram negative), *Escherichia coli* (gram negative) and two fungal strains viz. *Candida albicans* and *Aspergillus niger*.

The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water and was dispersed in 20ml volumes in to test tubes. The test tubes were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lb psig) for 15 minutes. The contents of tubes were poured aseptically in to sterile Petri dishes (90mm diameter) and allowed to solidify.

Muller Hinton agar medium was used to inoculate bacterial cultures and Sabouraud's dextrose agar medium was used for fungal cultures.

Graded concentration of the test compound should be prepared by serial dilutions and added in to an appropriate agar medium. A suitably diluted suspension of test organism was inoculated, in the form of tiny drops, on the surface of agar medium. After incubation presence/absence of the growth of organism on the agar medium was observed and from the results, the MIC of the test compound was calculated. Six test tubes were labeled 1 to 6. Dimethylformamide (DMF) was transferred 2 ml into tube-1 and 1 ml each remaining 5 tubes. All tubes were plugged with non-absorbent cotton and sterilized by autoclaving at 121°C for 20 minutes. 32 mg of the test compound was aseptically transferred and dissolved in tube-1. From this 1 ml of solution was transferred to test tube-2 and mixed well. This process is repeated 6 times and 1ml of solution from tube-6 was discarded. 15 ml molten Muller Hinton agar/fluid Sabouraud's dextrose agar was aseptically added into each test tube, gives concentration of compounds 1000, 500, 250, 125, 62.5 and 31.25 μ g/ml respectively. The tubes were mixed well aseptically poured into a sterile Petri dish and this medium was allowed to solidify. Each plate was divided into four quadrants. Each quadrant was inoculated with different test organism. 10 drops of suitably diluted suspension (10^6 Cfu/ml) of the test organisms (bacterial cultures on Muller Hinton agar or fungal cultures on Sabouraud's agar) was inoculated on the surface of agar medium, in the form of tiny droplets, using a sterile 1ml syringe with 24-gauge needle. A positive control was prepared in a similar manner except with the test compound not adding in to the agar medium. A negative control was prepared in a similar way except the text compound was not added and the tube was not inoculated with test organism. All plates were inoculated at an appropriate condition. Tubes inoculated with

bacterial cultures were incubated aerobically at 37°C for 24 h and tubes inoculate with fungal cultures were incubated aerobically at 25°C for 48 h. The tubes were kept under observation for the presence/absence of growth in the form of colonies.

RESULTS AND DISCUSSION

The structures of the compounds were established on the basis of IR, ¹H NMR, GCMS and elemental analysis. The synthesized compounds were assessed for the antibacterial activity against four bacterial strains, namely *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and antifungal activity was carried out on two fungal strains, namely *Candida albicans* and *Aspergillus niger* by minimum inhibitory concentration method. The antimicrobial screening results have been summarized in Table-2.

TABLE-2: ANTIMICROBIAL ACTIVITY OF N-[(2-SUBSTITUTED PHENYL)-4-OXO-1,3 - THIAZOLIDINE-3-YL]ISONICOTINAMIDES

No.	Minimum Inhibitory Concentration (MIC)					
	Bacteria				Fungi	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E.coli</i>	<i>C. albicans</i>	<i>A. niger</i>
3a	500	250	500	500	500	500
3b	500	250	500	250	500	500
3c	125	125	125	125	125	125
3d	250	500	250	250	500	500
3e	125	250	250	250	250	250

MIC in µg/ml. Antimicrobial activities of all the synthesized compounds were tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and two fungal strains *Candida albicans* and *Aspergillus niger*.

The results shows that compound 3c exhibited good antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* and compound 3e exhibited good antibacterial activity against *Bacillus subtilis* with MIC of 125 µg/ml. Compound 3a exhibited moderate activity against *Staphylococcus aureus*, compound 3b exhibited moderate activity against *Staphylococcus aureus* and *Escherichia coli*, compound 3d exhibited moderate activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and compound 3e exhibited moderate activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* with MIC of 250 µg/ml. Compound 3a exhibited significant activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*, compound 3b exhibited significant activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* and compound 3d exhibited significant activity against *Escherichia coli* and *Staphylococcus aureus* with MIC of 500 µg/ml.

Compound 3c exhibited good antifungal activity against *Candida albicans* and *Aspergillus niger* with MIC of 125 µg/ml. Compound 3e exhibited moderate activity against *Candida albicans* and *Aspergillus niger* with MIC of 250 µg/ml. Compound 3a, 3b and 3d exhibited significant activity against *Candida albicans* and *Aspergillus niger* with MIC of 500 µg/ml.

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