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Synthesis and Biological Evaluation of Some Novel Nicotinic Acid Derivatives

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ABSTRACT In the present study, six compounds of thiazolidinone derivatives of nicotinic acid were synthesized by reacting Schiff bases with thioglycolic acid and zinc chloride in ethanol. The Schiff bases were synthesized by treating various aromatic aldehydes with nicotinic acid hydrazide. Reaction between nicotinic acid and phosphorous pentachloride gives nicotinoyl chloride. Further nicotinoyl chloride is converted to nicotinic acid hydrazide by the action of hydrazine hydrate. Structure of these products has been established by IR, ^{1}HNMR and Mass spectral data. The *in vitro* antibacterial and antifungal activities of the compounds were evaluated by paper disc diffusion method. The Minimum Inhibitory Concentration (MIC) of the compounds was also determined by agar steak dilution method. Significant antimicrobial activities were observed for some compounds of the series. Compounds were also screened for their anti-inflammatory and analgesic activity. Some of them showed comparable activity as that of the standard drug used.

(Keywords: Thiazolidinone, antimicrobial, analgesic, anti-inflammatory)

INTRODUCTION

Thiazolidinone derivatives are reported to show variety of biological activities such as antiviral, antibacterial, antifungal, pesticidal, herbicidal, antiprotozoal, namatocidal, anti tubercular, anticancer, local anesthetic, anticonvulsant, antioxidant, anti-inflammatory and possible antimycotic properties [1,2, 3]. Nicotinic acid which belongs to water soluble vitamin B complex is also indicated is hyperlipidemia to lower triglycerides and cholesterol [4, 5]. Keeping in view the importance of the above heteryl nuclei and considering the scope to introduce nicotinoyl moiety into heterocyclic compounds it was thought worthwhile to undertake the synthesis of the titled compounds. The compounds have been identified on the basis of spectral studies.

MATERIALS AND METHODS

In the present study six different thiazolidinones were synthesized from nicotinic acid. Nicotinic acid on treatment with phosphorus pentachloride in presence of carbon tetra chloride yielded nicotinoyl chloride (1). Nicotinoyl chloride when treated with hydrazine hydrate gives nicotinic acid hydrazide (2). Nicotinic acid hydrazide was condensed with six different aldehydes to yield six different Schiff bases (3). Various thiazolidinones (A – F) were obtained by the cyclization of the Schiff bases with thioglycolic acid in the presence of ethanol and zinc chloride. The melting points were taken in open capillary tubes and are uncorrected. The **IR** spectra [6] of the compounds were recorded on **ABB BOMEN FTIR** spectrometer **MB 104** with potassium bromide pellets. **^{1}H NMR** spectra [6] in $CDCl_3$ or DMSO-d6 recorded on Burker AV **400 MHz** spectrometer. The chemical shift is reported as parts per million down fields from tetra methyl silane. Mass spectra were recorded on **GCMS QP 5000** Shimadzu. The purity of the compounds was checked by **TLC** on precoated aluminium plates of silica gel. The spots were visualized by UV light. The physical data are tabulated in Table 1.

Table 1. Physical Data of the Synthesized Compounds

Compounds	R_1	R_2	R_3	Molecular Formula	% Yield	M.P. ^oC	R_f Values
A	–H	–H	–H	$C_{15}H_{13}N_3O_2S$	70	297	0.72
B	–OH	–H	–H	$C_{15}H_{13}N_3O_3S$	65	278	0.81
C	–H	–H	–Cl	$C_{15}H_{12}N_3O_2SCl$	82	309	0.69
D	–H	–H	$–OCH_3$	$C_{16}H_{15}N_3O_3S$	75	240	0.77
E	–H	$–OCH_3$	–OH	$C_{16}H_{15}N_3O_4S$	70	310	0.79
F	–H	–OH	–H	$C_{15}H_{13}N_3O_3S$	68	300	0.74

R_f value was determined in mixture of DMF and DMSO (1:1)

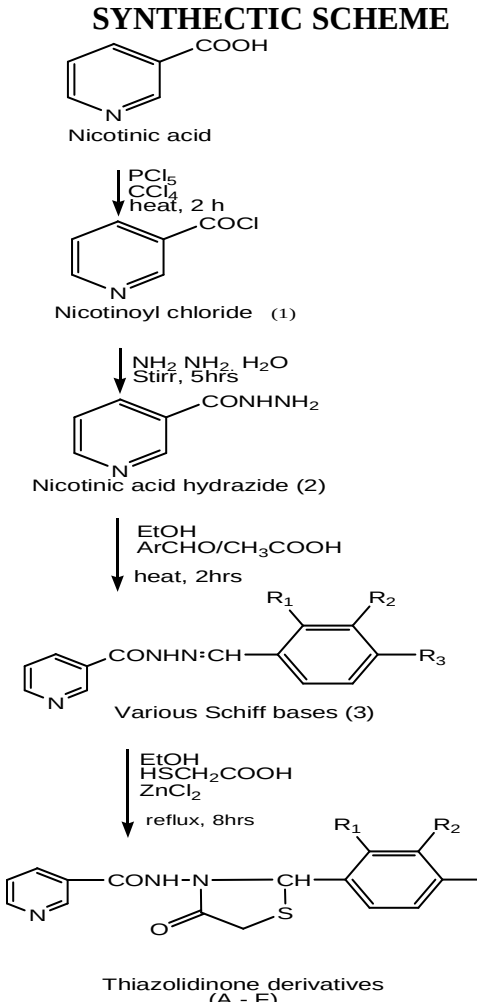

Figure 1. Reaction pathway showing the formation of thiazolidinones.

General Details of Synthesis of Compounds

Synthesis of nicotinoyl chloride (1)

A mixture of nicotinic acid (0.03 mol) and phosphorous pentachloride (0.05 mol) in anhydrous carbon tetra chloride (20 ml) was refluxed for 2 hours at 100^0 C. Solvent was distilled off and the solid acid chloride thus obtained was used for further reaction without any purification. Yield = 87.5%, m.p. = 188-194^0C.

Synthesis of nicotinic acid hydrazide (2)

To the acid chloride (0.03 mol) was added hydrazine hydrate (0.1 mol) drop wise at 0^0 C and the resultant mixture was stirred for 5 hours at room temperature. The solid was formed and it was washed with aqueous sodium bicarbonate (10%) and dried in vacuum. It was recrystallized using methanol. Yield = 78.2%, m.p. 148 –150^0C.

Synthesis of Schiff bases (3)

Nicotinic acid hydrazide (2) (0.01 mol) and appropriate aromatic aldehyde (0.01 mol) in ethanol (50ml) containing a few drops of acetic acid was heated gently for 1 hour at a temperature of 60^0C. The reaction mixture is then poured into ice cold water and it was filtered. The pure compound was obtained from DMF. (Six different aldehydes used were benzaldehyde, 2-hydroxy benzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde and 3 –hyroxybenzaldehyde).

Synthesis of various thiazolidinones (A – F)
A mixture of Schiff bases (0.01 mol) in ethanol and thioglycollic acid (0.01 mol) with a pinch of zinc chloride was refluxed on a steam bath for about 8 hours. The separated solids were crystallized from methanol to give compounds A to F. Unreacted thioglycollic acid was removed by the addition of saturated sodium bicarbonate solution.

IR, NMR and Mass spectral data of the synthesized compounds

***N-(4-oxo-2-phenylthiazolidin-3-yl) nicotinamide* (A)**
IR (KBr) cm^{-1}: 3045 (C-H(s)), 853 (C-H(b)), 1589 (C=C), 1493 (C=N), 1721 (C=O), 1141 (C=S), 1693 (CONH).
^{1}HNMR ($CDCl_3$)δ: 7.2-8.8 (9H, m, aromatic and hetero aromatic), 6.3 (1H,s, CONH), 4.4 (2H,s, CH_2), 3.4 (1H,s, CH).
MS: m/z 299(M^+), 237, 203, 147, 95, 81, 60,.68.

***N-(2-(2-hydroxyphenyl)-4-oxathiazolidin-3-yl) nicotinamide* (B)**
IR (KBr) cm^{-1}: 3051 (C-H(s)), 853 (C-H(b)), 1589 (C=C), 1491 (C=N), 3582 (Ar-OH), 1733 (C=O), 1142 (C=S), 1698 (CONH).
^{1}HNMR ($CDCl_3$)δ: 6.7-8.5 (8H, m, aromatic and hetero aromatic), 5.6 (1H, s, phenolic OH), 6.3 (1H, s, CONH), 4.4 (2H,s, CH_2), 3.3(1H,s, CH).
MS: m/z 315(M^+), 203, 148, 84, 68, 56, 237.

***N-(2-(4-chlorophenyl)-4-oxathiazolidin-3-yl) nicotinamide* (C)**
IR (KBr) cm^{-1}: 3049 (C-H(s)), 824 (C-H(b)), 1590 (C=C), 1488 (C=N), 815 (Ar-Cl), 1719 (C=O), 1141 (C=S), 1680 (CONH).
^{1}HNMR ($CDCl_3$)δ: 7.2-8.7 (8H, m, aromatic and hetero aromatic), 6.3 (1H, s, CONH) 4.4 (2H,s, CH_2), 3.3(1H,s, CH).
MS: m/z 333(M^+), 237, 220,149 88, 68, 56, 203.

***N-(2-(4-methoxyphenyl)-4-oxothiazolidin-3-yl) nicotinamide* (D)**
IR (KBr) cm^{-1}: 3048 (C-H(s)), 824 (C-H (b)), 1590 (C=C), 1493 (C=N), 1097 (Ar-OCH_3), 1715 (C=O), 1148 (C=S), 1097 (Ar-OCH_3), 1715 (C=O), 1141 (C=S), 1690.61 (CONH).
^{1}HNMR ($CDCl_3$)δ: 7.0-8.7 (8H, m, aromatic and hetero aromatic), 3.2 (3H, s, OCH_3), 6.3 (1H, s, CONH), 4.3 (2H,s, CH_2), 3.4(1H,s, CH).
MS: m/z 329(M^+), 203, 149, 88, 68, 56, 237.

***N-(2-(4-hydroxy-3-methoxyphenyl)-4-oxothiazolidin-3-yl) nicotinamide* (E)**
IR (KBr) cm^{-1}: 3032 (C-H(s)), 823 (C-H (b)), 1589 (C=C), 1493 (C=N), 3578 (Ar-OH), 1057 (Ar-OCH_3) 1750 (C=O), 1141 (C=S), 1680 (CONH).
^{1}HNMR ($CDCl_3$)δ: 6.6-8.6 (8H, m, aromatic and hetero aromatic), 3.3 (3H, s, OCH_3), 5.2 (1H s phenolic OH), 6.2 (1H, s, CONH), 4.3 (2H, s, CH_2), 3.3(1H,s, CH).
MS: m/z 237(M^+), 149, 76, 68, 54, 203.

***N-(2-(3-hydroxyphenyl)-4-oxothiazolidin-3-yl) nicotinamide* (F)**
IR (KBr) cm^{-1}: 3045 (C-H(s)), 824 (C-H (b)), 1589 (C=C), 1492 (C=N), 3585 (Ar-OH), 1731 (C=O), 1141 (C=S), 1681 (CONH).
^{1}HNMR ($CDCl_3$)δ: 6.5-8.8 (8H, m, aromatic and hetero aromatic), 5.5 (1H,s,. phenolic OH), 6.3 (1H, s, CONH), 4.3 (2H,s, CH_2), 3.3(1H,s, CH).
MS: m/z 315(M^+), 237, 203, 95, 81, 56, 68.

BIOLOGICAL INVESTIGATION
The *in vitro* antibacterial and antifungal activities of the compounds were evaluated at 25μg/ml, 50μg/ml and 75 μg/ml concentration by paper disc diffusion method [7]. Ciprofloxacin 50μg/ml and Ketaconazole 50μg/ml were used as standard. DMSO was used as solvent control. The Minimum Inhibitory Concentrations of the compounds were also determined by agar steak dilution method. The analgesic activity (Chemical writhing induced by acetic acid in mice) and anti-inflammatory activity (Carrageenan induced paw edema in rats) were performed for five thiazolidinone derivatives. Acute oral toxicity test was performed for all the synthesized compounds as per Organization of Economical Co-operation and Development (OECD) guidelines- 423.

ANTIMICROBIAL ACTIVITY
The antibacterial activity of the synthesized compounds was tested against *Staphylococcus aureus*, *Staphylococcu epidermidis*, *Klebsiella pneumonia* and *Escherichia coli* using nutrient agar medium. The antifungal activity of the compounds was tested against *Candida albicans* and *Aspergillus niger* using sabouraud dextrose agar medium.

Paper Disc Diffusion Medium

A suspension of the organisms were added to sterile nutrient agar media at 45°C and the mixture was transferred to sterile Petri dishes and allowed to solidify. Sterile discs 6mm in diameter (made from Wattmann filter paper previously sterilized in U.V. lamp) dipped in specified concentration solutions of synthesized compounds and standard were placed on the surface of agar plates. A disc dipped in DMF was also used as control. The plates were left for 1 hour at room temperature as a period of preincubation diffusion to minimize the effects to variation in time between the applications of the different solutions. The plates were then incubated at 37 ± 1°C for 24 hours and observed for antibacterial activity. The diameters of zone of inhibition were measured and compared with that of the standard, the values were tabulated. Ciprofloxacin (50 μg/disc) and Ketoconazole (50 μg/disc) were used as standard for antibacterial and antifungal activity respectively. The observed zone of inhibition is presented in Table 2.

Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) of the test compounds were determined by agar streak dilution method. 1 mg/ml stock solution of the synthesized compounds were made using DMF as the solvent. From this stock solution, required quantities of drug solutions were mixed with the known quantities of molten sterile agar media aseptically to provide the following concentrations 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 100 μg/ml. About 20 ml of the media containing the drug was dispensed into each sterile Petri dish. Then the media were allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking all the plates were incubated at 37 ± 1°C for 24 hours/48 hours for bacterial and fungus activity respectively. Then the plates were observed for the growth of microorganisms. The lowest concentration of the synthesized compounds inhibiting the growth of the given bacteria/fungus was considered as minimum inhibitory concentration (MIC) of the test compounds against that bacteria or fungi on the plate. The MIC values of each compound against various bacteria and fungus were tabulated in Table 2.

Table 2. Antimicrobial activity of compounds A – F

Compd.	Zone of inhibition (mm)						MIC (μg/disc)					
	Antibacterial activity†				Antifungal activity†		Antibacterial activity†				Antifungal activity†	
	S.A.	***S.E.***	***K.P.***	***E.C.***	***A.N.***	***C.A.***	***S.A.***	***S.E.***	***K.P.***	***E.C.***	***A.N.***	***C.A.***
A	+	+	++	++	+++	++	+	++	+	+	+	+
B	++	++	+++	++	+++	++	+	+	+	+	+	+
C	++	++	++	++	+++	++	+	++	+	+	+	+
D	++	++	+++	+++	++	++	+	+	+	+	+	+
E	+	++	++	+	+++	++	+	+	+	++	+	+
F	++	++	++	+	++	++	+	+	++	+	+	+
C.P.	+++	+++	++++	++++	-	-						
K.C.	-	-	-	-	++++	+++						
C.	-	-	-	-	-	-						

† S.A. - *Staphylococcus aureus*; S.E. – *Staphylococcus epidermidis*; K.P. – *Klebsiella pneumonia*; E.C. – *Escherichia coli*; A.N. – *Aspergillus niger*; C.A. – *Candida albicans*;
C.P. – Ciprofloxacin (50 μg/ml); K.C. – Ketaconazole (50 μg/ml); C – Control
Concentration of the synthesized compounds: 50 μg/ml
Zone of inhibition: 10-15 mm = +; 16-20 mm = ++; 21-25 mm = +++ and 25 < = ++++.

PHARMACOLOGICAL EVALUATION

Acute Oral Toxicity

Acute oral toxicity [9] was performed as per OECD – 423 guidelines (acute toxic class method). Swiss albino mice (n=3) of either (sex selected by random sampling technique) were used for the study. The animals were kept fasting for 3-4 hours providing only water, after which the test compounds (suspended in 1% CMC) were administered orally at the dose level of 5 mg/kg by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. In the present study, mortality was not observed and the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg.

Analgesic Activity

The animals were divided into seven groups of six animals each. SCMC 1% w/v was administered orally as vehicle control to Group I. Group VII received 100 mg/kg body weight of aspirin intraperitonially which served as standard. Group II – VI was administered with synthesized compounds A – E orally at a dose of 70 mg/kg body weight. Writhing was induced by an intraperitonial injection of 0.1 ml/10g body weight of 0.6% v/v of acetic acid. The test substances for all the groups were given one hour before the acetic acid injection. Immediately after the administration of acetic acid the mouse was isolated in an individual cage and the writhing was observed for 20 min from the onset of writhing action [10, 11]. The % protection was tabulated in Table 3.

Anti inflammatory Activity

Rats of either sex were randomly divided into seven groups. Each group composed of six animals. Diclofenac in the dose of 50 mg/kg was administered intraperitonially as standard to Group-VII. CMC 1% w/v was administered intraperitonially as vehicle control to Group-I. Groups II – VII received 70 mg/kg body weight of Compounds A – F orally. Half an hour after dosing the animals with drug, the rats received at the sub plantar region of the left hind paw 0.1 ml of 1% carrageenan suspension. While the left paw of the induction of the inflammatory agent the right and left hind paw volume displacement was measured by using mercury plesthmograph. This constituted zero minute reading. The measurements of both the feet volumes were taken at 1, 2, 3, 4 and 5 hrs [12]. The % protection was tabulated in Table 4.

Table 3. Acetic acid induced writhing (Analgesic activity)

Group	Treatment	No of writhing in 20 minutes	% protection
I	1% SCMC	60.07 ± 2.89	**-**
II	A	$25.67 \pm 1.56^{*}$	59%
III	B	$28.66 \pm 1.31^{@}$	52%
IV	C	$27.34 \pm 1.27^{@}$	54%
V	D	$41.32 \pm 2.17^{@}$	31%
VI	E	$30.16 \pm 1.58^{@}$	49%
VII	Aspirin	$12.5 \pm 0.59^{\#}$	79%

All values are mean ± SEM from 6 animals in each group.
Comparison Groups II, III, IV, V and VI vs. Group I.
$^{@}P < 0.05$, $^{*}P < 0.01$, $^{\#}P<0.001$ ^{NS}- Non signifiant
.

Table 4. Effect of synthesized compounds on carrageenan induced paw edema (Anti-inflammatory activity)

Group	Treatment	Edema volume (ml) 1 hr	2 hr	3 hr	4 hr	5 hr
I	1% CMC	0.18 ± 0.007	0.26 ± 0.006	0.32 ± 0.008	0.36 ± 0.011	0.42 ± 0.014
II	A	0.18 ± 0.011^{NS} (3.22 %)	0.23 ± 0.014$^{@}$ (12.54 %)	0.22 ± 0.016* (31.48 %)	0.20 ± .011* (44.44 %)	0.17 ± 0.023* (55.83 %)
III	B	0.17 ± 0.020^{NS} (5.01 %)	0.22 ± 0.017* (16.34 %)	0.21 ± 0.018* (35.58 %)	0.18 ± .014* (49.99 %)	0.15 ± 0.009* (62.50 %)
IV	C	0.19 ± 0.014^{NS} (6.14%)	0.22 ± 0.018$^{@}$ (10.56%)	0.21 ± 0.011* (37.24%)	0.19 ± .018* (47.1%)	0.17 ± 0.016* (56.6%)
V	D	0.2 ± 0.011^{NS} (2.96%)	0.23 ± 0.014^{NS} (6.49%)	0.22 ± 0.009* (33.5%)	0.21 ± .008* (40.0%)	0.17 ± 0.018* (47.5%)
VI	E	0.19 ± 0.014^{NS} (7.76%)	0.20 ± 0.016$^{@}$ (15.98%)	0.18 ± 0.008$^{@}$ (47.05%)	0.16 ± .015* (55.5%)	0.13 ±0.011* (67.5%)
VII	Diclofenac	0.16 ± 0.004$^{@}$ (10.38 %)	0.19 ± 0.006* (25.22 %)	0.18 ± 0.007* (42.74 %)	0.15 ±0.016* (56.48 %)	0.12 ± 0.018* (72.16%)

All values are mean ± SEM from 6 animals in each group.
% inhibition shown in parenthesis.
Comparison Groups II, III, IV, V and VI vs. Group I.
$^{@}$ $P < 0.01$, * $P < 0.001$, ^{NS}- Non significant.

RESULTS AND DISCUSSION

All the synthesized compounds were evaluated for *in vitro* antibacterial and significant antifungal activities. Among the compounds, compounds B and D pocess significant activity against bacterial organisms whereas C showed very less activity. Other compounds showed moderate antibacterial activity. Compounds C and E, showed Zone of inhibition (for 50μg/ml) and MIC of the synthesized compounds have been summarized in Table 2.

All the synthesized compounds did not cause mortality up to 2000mg/kg in acute oral toxicity (OECD-423 guidelines) and were considered at safe. The synthesized compounds A, B and C significantly ($p< 0.01$ and $p< 0.05$ respectively) reduced the number of writhing which showed moderate analgesic activity than other compounds when compared to that of standard (Table 3). Compounds B and E show significant reduction in paw edema when compared to the standard. Compound E showed 67.5% of protection where as compound B showed 62.5% of protection (Table 4).

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