WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 9, 2102-2111. Research Article ISSN 2277– 7105

CONVENTIONAL METHOD OF BIOACTIVE SYNTHESIS OF N-PHENYL NICOTINAMIDE DERIVATIVES PROMOTED BY DCC

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Article Received on
06 April 2023,

Revised on 27 April 2023,
Accepted on 17 May 2023

DOI: 10.20959/wjpr20238-28316

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ABSTRACT

In the present study, conventional method of bioactive synthesis of N-phenyl nicotinamide derivatives 4). These derivatives (4) can be prepared from nicotinoylchloride (2) with various substituted anilines (3) in the presence of DCC in ethanol at reflux. The nicotinoylchloride (2) can be obtained by nicotinic acid with thionyl chloride in the presence of MDC at reflux. All prepared compounds were characterized via FT-IR spectroscopy; some of them were characterized by ^{1}H-NMR and ^{13}CNMR spectroscopy. These new N-phenyl nicotinamide derivatives were tested pharmacological activities in microbial activity. Some of tested compounds showed strong activity while the other showed moderate against.

KEYWORDS: ^{1}H-NMR and ^{13}CNMR spectroscopy.

1. INTRODUCTION

The amide bond is of particular importance, not only for its key function in peptide structures, but also its role in a major of natural and synthetic small molecules and polymers.[1,2] Amide bond formation is traditionally achieved through the activation of the carboxylic acid partner using a greater-than-stoichiometric quantity of some complex activating agent such as carbodimide + additives (HOBt, HOAt or Oxyma), Phosphonium or guanidinium salts, etc. Thus generating a large amount of bi products, whereas amide formation is 'just' about the elimination of one molecule of water. In 2006, a round table dedicated to the improvement of green chemistry research ranked "amide formation avoiding poor atom economy reagents" as a top priority.[3] This point is even more crucial, as the formation of amides from carboxylic acid and amines is by far the most used reaction in medicinal chemistry.[4] This review intends to highlight more recent progress in catalytic

formation of amide bonds from amines, carboxylic acids and esters (Scheme 1).[5–7] These catalytic amidation strategies were recently covered by Sheppard, Wang and Perrin, who focused on green aspects, perspectives and peptide synthesis.[8–10] General preparation of amide bond formation that do not focus on catalytic direct approaches have also appeared.[11,12] Catalytic redox reactions starting from aldehydes or alcohols are beyond the scope of this review and, consequently, will not be reported herein.[12] C–H catalytic amidation reactions from nitrene precursors, which cannot be considered as direct amidation of amines, have been recently covered in comprehensive reviews and thus will not be addressed here.[13] This review aims to focus on mechanistic and practical aspects and to discuss substrate tolerance and racemization (whenever relevant).

2. MATERIALS AND METHODS

2.1. General

All chemicals, solvents, and media were procured from Sigma Aldrich. All commercial chemicals were used without further purification before use, reactions were continuously monitored by thin layer chromatography (TLC) on silica gel-(G60 F254, Merck) of 0.5 mm thickness, visualizing with ultraviolet light (256 and 367 nm), or with iodine vapour. The melting points of the titled derivatives were determined using a Buchi B-540 capillary apparatus. NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) respectively in solvents like $CDCl_3$ and chemical shifts were referenced to the solvent residual signals with respect to tetramethylsilanes. Standard abbreviations are used to represent signals multiplicities for 1H NMR spectrum s - singlet, d - doublet, t - triplet, q - quartet, m - multiplet. The reaction temperature was monitored by ruby thermometer. Mass spectra were recorded on a Shimadzu GC-MS-QP-2010 mass spectrometer in EI (70eV) model using direct inlet probe technique and m/z were reported in atomic units per elementary charge.

2.3. Experimental

2.3.1. The general procedure of Nicotinoyl chloride

Take dry and clean four necks RBF. The starting material niconicacid in dissolved in MDC and Thionyl chloride added drop wise in above solution into RBF at 5-10^{0}C and also fitted on the magnetic stirrer possesses hot plate. The reaction mixture continuous carried the reaction for 2 hrs. at 40^{0}C. The progress of the reaction monitored by the TLC (EtOAc: n-hexane = 6:4). After completion of the consumed all reactants, cooled the reaction mixture at

RT. The crude neutralized with as saturated solution of sodium bicarbonate and poured in ethyl acetate. The separated the organic layer and washed with water and separated the ethyl acetate layer. An organic layer can be distilled off under vacuums and solid compound obtained.

Characterization of Nicotinoyl chloride (2)

Yield: 90%, White solid, m.p. (°C): 185; IR (KBr, νmax, cm-1): 3045 (=C-H aromatic), 2978 & 2917 (-C-H aliphatic), 1608 & 1545 (C=C aromatic),^{1}H NMR (400 MHz, $CDCl_3$) δppm: 8.475 (s, 1H,Pyridine), 8.310 (d, J=8.0Hz,1H,Pyridine), 7.824 (d, J=7.4Hz, 1H,pyridine), 7.495 (s, 1H, pyridine), ^{13}C NMR (100 MHz, $CDCl_3$) δppm:149.22, 145.65, 135.24, 133.04, 129.12, LCMS (m/z): 143.21(M+2); Molecular formulae: C6H4CNO. Elemental Analysis: Calculated: C- 50.91; H- 2.85; N- 9.90; Obtained: C-50.84; H-2.83; N-9.68.

2.3.2. The general procedure of N-phenylnicotinamide

Take dry and clean four necks RBF. The charge the nicoticchloride with methylene dichloride at 5-10^{0}C temperature which is also fitted on the magnetic stirrer possesses hot plate. The charge a mixture of substituted aromatic anilines into RBF at mixture carried out 40^{0}C. Before start the reaction, the strong base such as DCC and triethyl amine added into the reaction mixture and reaction continued in 5hrs at same temperature and monitored by TLC (ethyl acetate and n-hexane). After the completion of the reaction, crude poured in cold water and add 10 mL of 10% saturated solution of sodium bi carbonate added into the solution and charge with ethyl acetate. The ethylacetae layer separated and washed with solution of Brain. Finally separated the ethylacetae layer and distilled off. The desired product separated by column chromatography and also recrystallized with ethanol N-phenylnicotinamide (4a-e).

Characterization of Nicotinoyl chloride (4a-e)

2.3.2.1. N-phenylnicotinamide (4a)

Yield: 86%, colour: brown solid, m.p. (°C): 246–248. IR (KBr, νmax, cm-1): 3374 (N-H amidic), 3074 (=C-H aromatic), 2965 & 2911 (-C-H aliphatic), 1689 (-CONH amidic ketone), 1612 & 1588 (C=C aromatic), 1305 (=C-O-C asymmetric), 1178 (C-N), 1012 (=C-O-C symmetric), 813 (p-disubsituted aromatic), 1H NMR (400 MHz, $CDCl_3$) δppm: 10.156(s, 1H, -CONH), 8.536 (s, 1H,Pyridine), 8.223 (d, J=8.4Hz,1H,Pyridine), 7.956 (d, J=8.0Hz, 1H,pyridine), 7.647-7.512 (m, 2H, Ar-H), 7.486 (s, 2H, pyridine), 7.327-7.286 (m, 2H, Ar-H), 13C NMR (100 MHz, CDCl3) δppm: 165.81, 145.22, 140.65, 135.13, 133.49,

129.46, 128.77, 128.25,126.29, 122.88. LCMS (m/z): 199.28(M+H); Molecular formulae: $C_{12}H_{10}N_2O$. Elemental Analysis: Calculated: C-72.71; H- 5.09; N- 14.13; Obtained: C-72.70; H-5.08; N- 14.21.

2.3.2.2.2. N-(4-methoxyphenyl) nicotinamide (4b)

Yield: 92%, pale brown solid, m.p. (°C): 254–256. IR (KBr, νmax, cm-1): 3355 (N-H amidic), 3071 (=C-H aromatic), 2972 & 2904 (-C-H aliphatic), 1688 (-CONH amidic ketone), 1611 & 1582 (C=C aromatic), 1307 (=C-O-C asymmetric), 1165 (C-N), 1017 (=C-O-C symmetric),788 (p-disubsituted aromatic), 1H NMR (400 MHz, $CDCl_3$) δppm: 10.157(s, 1H, -CONH), 8.746 (s, 1H,Pyridine), 8.402 (d, J=7.6Hz,1H,Pyridine), 7.584 (t, J=8.0Hz, 1H,pyridine), 7.542-7.273 (m, 4H, Ar-H),3.742(s,3H,CH3);13CNMR(100 MHz, CDCl3) δppm: 166.76,155.08, 145.66, 144.07, 136.66, 131.08 129.46, 127.77, 124.65, 117.76. LCMS (m/z): 228.49(M^+); Molecular formulae: $C_{13}H_{12}N_2O_2$. Elemental Analysis: Calculated: C-68.41.71; H- 5.30; N- 12.27; Obtained: C-68.35; H-35.22; N- 12.27.

2.3.2.3. N-(4-bromophenyl) nicotinamide (4c)

Yield: 90%, reddish brown solid, m.p. (°C): 272–274. IR (KBr, νmax, cm^{-1}): 3348 (N-H amidic), 3070 (=C-H aromatic), 2978 & 2917 (-C-H aliphatic), 1679 (-CONH amidic ketone), 1608 & 1586 (C=C aromatic), 1305 (=C-O-C asymmetric), 1162 (C-N), 1018 (=C-O-C symmetric),784 (p-disubsituted aromatic), 1H NMR (400 MHz, $CDCl_3$) δppm: 10.172(s, 1H, -CONH), 8.757 (s,1H,Pyridine), 8.423 (d, J=8.4Hz,1H,Pyridine), 8.184(d,J=7.2Hz, Pyridine), 7.426 (t, J=7.2Hz, 2H,pyridine), 7.523-7.293 (m, 4H, Ar-H), 13C NMR (100 MHz, CDCl3) δppm: 167.65,146.74, 145.33, 137.94, 133.94, 129.46, 128.96, 128.42, 127.37, 112.65. LCMS (m/z): 277.48(M+2); Molecular formulae: $C_{12}H_9N_2OBr$. Elemental Analysis: Calculated: C-52.01; H- 3.27; N- 10.11; Obtained: C-51.94; H-3.25; N- 10.19.

2.3.2.4. N-(4-cyanophenyl) nicotinamide (4d)

Yield: 87%, brown solid, m.p. (°C): 247–249. IR (KBr, νmax, cm^{-1}): 3348 (N-H amidic), 3065 (=C-H aromatic), 2974 & 2918 (-C-H aliphatic), 1677 (-CONH amidic ketone), 1609 & 1577 (C=C aromatic), 1308 (=C-O-C asymmetric), 1172 (C-N), 1021 (=C-O-C symmetric),787 (p-disubsituted aromatic), 1H NMR (400 MHz, $CDCl_3$) δppm: 10.215(s, 1H, -CONH), 8.727 (s,1H,Pyridine), 8.526 (d, J=8.8Hz,1H,Pyridine), 8.196 (d,J=7.6Hz,1H, Pyridine), 7.804-7.645 (m, 2H, Ar-H), 7.614(t, J=7.2Hz, 2H,pyridine), 7.527 (d, J=7.6Hz,2H,Ar-H); 13C NMR (100 MHz, CDCl3) δppm: 167.99,147.76, 145.65,140.38, 136.08, 130.14, 127.62, 125.35, 119.42, 109.62; LCMS (m/z): 224.08(M+2); Molecular

formulae: $C_{13}H_9N_3O$. Elemental Analysis: Calculated: C-69.95; H- 4.06; N- 18.82; Obtained: C-69.95; H-4.04; N- 18.91.

2.3.2.5. N-(4-nitrophenyl) nicotinamide (5c)

Yield: 88%, brown solid, m.p. (°C): 268–270. IR (KBr, νmax, cm^{-1}): 3368 (N-H amidic), 3074 (=C-H aromatic), 2981 & 2907 (-C-H aliphatic), 1672 (-CONH amidic ketone), 1612 & 1576 (C=C aromatic), 1310 (=C-O-C asymmetric), 1170 (C-N), 1023 (=C-O-C symmetric),780 (p-disubsituted aromatic), 1H NMR (400 MHz, $CDCl_3$) δppm: 10.325(s, 1H, -CONH), 8.723 (s,1H,Pyridine), 8.514 (d, J=7.6Hz,1H,Ar-H), 8.168 (d,J=8.0Hz,1H, Pyridine), 8.045-7.813 (m, 2H, Ar-H), 7.523(t, J=7.6Hz, 2H,pyridine); 13C NMR (100 MHz, CDCl3) δppm: 167.74,147.33, 145.62,140.32, 135.65, 130.66, 127.62, 125.35, 120.62;LCMS (m/z): 243.17(M+); Molecular formulae: $C_{12}H_9N_3O_3$. Elemental Analysis: Calculated: C-59.26; H- 3.73; N- 17.28; Obtained: C-59.19; H-3.71; N- 17.36.

3. Biological Activities

3.1. Antibacterial activity

Compounds (4a-e) were examined for their antibacterial activity by using well diffusion technique of Agar medium. This media was used for the study of bacterial strains broth culture. In the sterilized condition, from the preparation culture, a small amount of culture was taken into 10–15 mL sterile normal saline (0.9% NaCl solution) and was thoroughly mixed. Approximately 0.5 ml of inoculums and melted agar cooled to 45 °C was added to the sterilized Petri dish and was allowed to solidify. Bacterial strains incubated for maintained 24 hrs. and were uniformly smeared on sterile nutrient agar medium in each using petri dishes sterile L-Shaped glass rod. Four uniform wells of 6 mm diameter were bored using cork borer to accommodate 50 µL of the solution in each well. Test samples were dissolved in DMSO, a negative control and Chloramphenicol (10 µg/50 µL) a positive control was taken as a standard drug, purchased from Himedia, Mumbai. The concentration of 50 µg/50 µL per well was used to assay the activity. Sterile micropipette tips were used to load the wells with the right amount of sample, control, and standard. After inoculation plates were incubated at 37 °C for 36 h. After the incubation period, zone of inhibition diameter for each well was measured in mm. The MIC and experiment performed in triplicates the average values are tabulated in Table-I.

3.2. Antifungal Activity

Antifungal activities of all N-phenylnicotinamide derivatives towards two mold fungi were studied, viz. Candida albicans Aspergillus flavus (human pathogen) (mold). The assay the antifungal activity of the synthesized compounds was used poisoned food technique method (Mohan N R et al., 2014) and Nystatin (10µg/disc) as a standard fungicide. Basal medium used for test fungi was Potato Dextrose Agar (PDA) it was prepared by using Potato, Dextrose, and Agar. First, crushed potatoes we boiled in water to get potato soup. The filtered soup has been mixed up with Dextrose and Agar. It is then sterilized by autoclave at 121^0 C for 30 minutes and was cooled (~ 45 C) and 15 mL of sterilized melted PDA medium was poured into each sterilized petri dish. After solidification, small portions of the mycelium of each fungus were inoculated carefully on each PDA plate with the help of sterilized L-shaped rod. Plates were incubated at $(25 \pm 2)^0$ C for five days. Four uniform wells with 6 mm diameter were bored using sterile cork borer to accommodate 50 µL of the solution in each. Samples were dissolved in dimethyl sulfoxide (DMSO) a negative control, Nystatin a positive control was taken as a standard drug, purchased from Himedia, Mumbai. The concentration of 50µg/50µL per well was used to assess the activity. Sterile micropipette tips were used to load the wells with the right amount of sample, control, and standard. The plates were then kept at 40^0 C for 24 h to provide sufficient time too diffuse over a considerable area of the plates. After 24h plates were incubated at 25^0 C for 48h. After the incubation period, the Diameter of the zone of inhibition in mm was measured for each well. The MIC and experiment performed in triplicates the average values are tabulated in Table II.

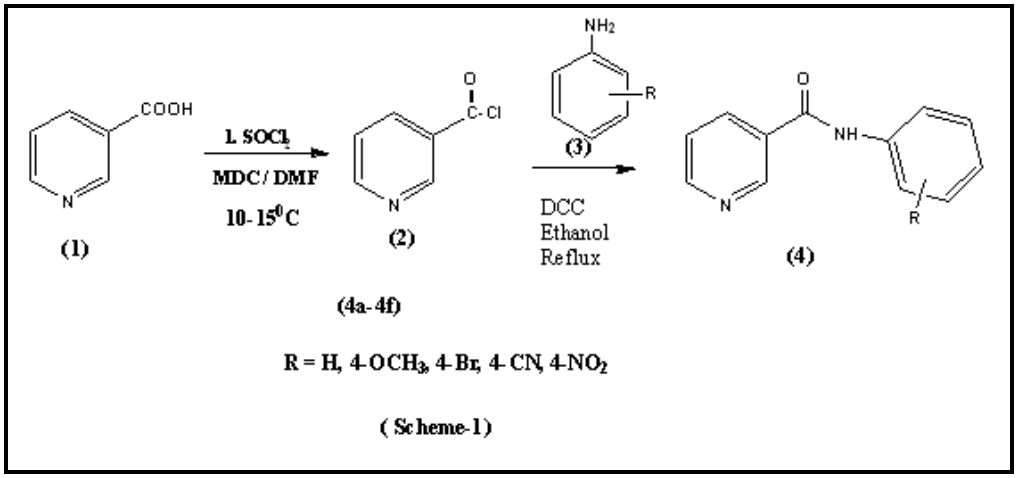
4. RESULTS AND DISCUSSION

4.1. Chemistry

Initially, in the present study, conventional method of bioactive synthesis of N-phenyl nicotinamide derivatives (4). These derivatives (4) can be prepared from nicotinoylchloride (2) with various substituted anilines (3) in the presence of DCC in ethanol at reflux. The nicotinoylchloride (2) can be obtained by nicotinic acid with thionyl chloride in the presence of MDC at reflux.

The results were indicated to yields derivatives that the aromatic anilines bearing both electron- releasing and electron-attracting groups were used and obtained the desired products in good yields. The advantages of the catalyst having some important features for the reaction conditions such as the simple work-up procedure, shortest reaction time,

excellent product yields, and purification of products by non-chromatographic methods. It is particularly observed that various substituted aromatic amines possess electron-donating or electron-donating withdrawing substituents in para-positions lead good yield of the product. Here, we have observed that the reaction of aromatic amines having electron-withdrawing groups was rapid as compared to the reaction of aldehydes having electron donating groups.(Scheme-1):



The structures of the desired compounds were characterized by 1HNMR, 13C NMR, mass spectral and elemental analyses. 1H NMR spectrum showed two singlets at 10.325 which were due to presence of HN-CO amide respectively. Further, aromatic proton on titled derivatives reveals that the ring resonated at δ 8.873 and 6.845 and also δ 167.74ppm which confirm the structure. The mass spectrum of 4c exhibited molecular ion peak at m/z : 277.48 (M+2); Molecular formulae: $C_{12}H_9N_2OBr$.

4.2.1. Antibacterial activity

The in vitro antibacterial activity of the desired compounds (4a-e) was compared with standard" Streptomycin'' as collected in (Table-I). As indicated in Table-I, most of the synthesized derivatives generally exhibited potent activity against all the tested bacterial strains. The derivatives "4c" showed excellent antibacterial potent activity against gram-positive bacterial strains viz; E.coli, A. and gram negative bacterial strains viz; B.subtilis, and S. aureus respectively due to such compounds possesses halogen atoms. The derivatives "4b" showed good active potential against bacterial strains. The compounds"4a, 4d and 4e"showed moderate activity against bacterial strains due to compounds having highly electron donating groups. These results indicated that the compounds having electron withdrawing groups

showed good activity than the compounds having electron donating groups. The derivatives containing halogen atoms showed excellent active potential against bacterial strains.

Table-I: Antibacterial activity of the newly synthesized compounds (4a-e).

Zones of inhibition (mm) of compounds (4a–e) against tested bacterial strains

Compound	Anti-Bacterial Activity			
	Gram(+ ve) bacteria		Gram(- ve) bacteria	
	E. coli	P. aureoginosa	B.subtilis	S. aureus
4a	04	09	05	07
4b	19	20	19	17
4c	25	26	22	21
4d	10	11	11	09
4e	07	05	07	06
Streptomycin	30	30	27	27
DMSO				

Streptomycin was used as standard. a 100 lg/mL of compound in each well.

Values are average of three readings.

4.2.2. Antifungal activity

The in vitro antifungal activity of the desired compounds (4a-e) was compared with standard drug" Ketonozole.'' as collected in (Table-II). The in vitro antifungal activity of the tested derivatives (4a-e) was investigated against A. Niger, A. favas and C.albicans using agar well diffusion assay and zones of inhibition of the test derivatives were expressed in mm as shown in Table-II. Compounds "4c" showed high active potential activity against the fungal strain. The compound having "4b" was found to be good active potential against tested fungal strain. Compounds 4a, 4d, and 4e have demonstrated significant antifungal activity comparable to standard. From the results it is reveals that most of the tested derivatives exhibited significant activity and few are moderately active as shown in Table -II. The remaining derivatives exhibited moderate potent activities against Aspergillusfavus. These results reveals that the compounds possess electron donating groups exhibited moderate activity while the compounds having electron attracting groups showed good against the fungal stains.

Table-II: Antifungal activity of the synthesized compounds (4a-e)

Zones of inhibition (mm)a of compounds (4a–e) against tested fungal strains:

Entry	Anti-Fungal Activity		
	Aspergillus Niger	Aspergillusfavus	Candida albicans
4a	06	07	06
4b	13	15	12
4c	18	18	17

4d	10	12	10
4e	08	08	09
Ketonozole	22	22	22
DMSO			

5. CONCLUSIONS

In conclusion, we have reported a direct synthesis of conventional method of bioactive synthesis of N-phenyl nicotinamide derivatives promoted by DCC. These N-phenyl nicotinamide derivatives can be prepared from nicotinoylchloride with various substituted anilines in the presence of DCC in ethanol at reflux. The nicotinoylchloride can be obtained by nicotinic acid with thionyl chloride in the presence of MDC at reflux. The reaction proceeds at normal temperature in the absence of any metallic catalyst and gives excellent selectivity (less than 92In this context, the proposed route gives access to N-phenyl nicotinamide derivatives without salt production and water as by-product. Furthermore, finally, these results gave us insights on the potential intermediates but further studies will be necessary to fully describe the mechanism of this transformation.

6. ACKNOWLEDGEMENTS

Authors are very thankful to PRISM PG&DG College and they were for providing all the necessary facilities.

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