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Synthesis and characterization of some new twin drugs having substituted pyridines

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ABSTRACT

There are many substituted pyridines having simple structures and greater pharmacological importance e.g. Nicotinamide, Nicotinic acid, 4- Amino pyridine and Isoniazid etc. Two drugs can be given simultaneously to the patient in the form of Twin Drugs in order to minimize the dose and side effects. Because of increase in lipophilicity, bioavailability is increased. Taking the same principle various non-identical twin drugs have been synthesized. Nicotinic acid has been combined with Fampridine (1) and Isoniazid (2), all used in the treatment of multiple sclerosis. Nicotinic acid has been combined with Salicylic Acid (3), both used in the treatment of atherosclerosis. PARP inhibitor i.e. Nicotinamide has been combined with COX inhibitor i.e. Aspirin(4),having anti-inflammatory activity. Partition co-efficient was also determined which showed that by making twin drugs, Lipophilicity and hence bioavailability is increased and dosing frequency and side effects are decreased.

Keywords: Nicotinamide, PARP, Twin drugs.

INTRODUCTION

Substituted pyridines are those in which there is substitution on the pyridine ring. Substitution can occur at any position of the pyridine ring, it may be complex or simple. There are many substituted pyridines having simple structures and greater pharmacological importance. Eg. Nicotinamide and Nicotinic acid. Another example of substituted pyridine is Isonicotinic acid hydrazide (Isoniazid) which is used in the treatment of tuberculosis and multiple sclerosis. It combines with pyridoxal or pyridoxal phosphate to form hydrazones, as a result, it is a potent inhibitor of pyridoxal kinase. Isoniazid thus appears to exert its anti-Vitamin B_6 effect primarily by inhibiting the formation of the coenzyme form of the vitamin [7,8]. Other substituted pyridine is 4-Amino Pyridine. It is also called as Fampridine. It is used in the treatment of multiple sclerosis. It is a potassium channel blocker. Normally single drug is used for the treatment of a particular disease. Sometimes at that particular dose, it shows some side effects. So in order to minimise the side effects, one has to decrease the dose of that drug and give another drug in combination with that drug having the similar effect. Mostly the side effects are dose dependent. So if dosage is decreased, the side effects are automatically reduced. In marketed tablets two powders of different drugs are combined. During this combination the lipophilicity of the drugs remain as such and it is not increased. But when they are given in the form of derivative, lipophilicity is increased because there is an increase in the number of carbon atoms and hence bioavailability is increased. The dosing of both the drugs will decrease because two drugs are given simultaneously and show synergistic effect.

When two drug molecules are joined together by covalent bonding, these are called as Twin Drugs. These are covalently bonded in such a way that the bond is easily broken in the body. Such twin products can be considered as transport forms from which the drug molecules combined are released before or after absorption in the body.

TWIN DRUGS may be **IDENTICAL or NON IDENTICAL**

Identical twin drugs- are those which are made up of two same drug molecules by covalent bonding [9].

e. g. Tetracycline ⟶ Ditetracycline(Dibiomycin)
Quinine ⟶ Diquinine Carbonate(Aristochin)
Salicylic acid ⟶ Salicyl salicylate, Salicil, Diaspirin
Isoniazid ⟶ Methylene bis isoniazid(Metazid)

Non Identical twin drugs- This class consist of drugs, which have different structural moieties. Upon administration, the non identical twin drugs are metabolised into two structural moieties, which can have identical or non identical pharmacological profiles [9]

Examples of non identical twin drugs are:

Streptomycin +Isoniazid ⟶ Streptoniazid (anti infectious)
Aspirin + Quinine ⟶ Quinineacetyl salicylate (Apochin-Analgesic)
Salicylic acid + Acetaminophen ⟶ Acetaminosalol (Cetosal -Analgesic)
Salicylaldehyde + Isoniazid ⟶ Saliniazid (Acozid- Anti infectious)
Aspirin + Paracetamol ⟶ Benorylate (Analgesic)

Nicotinamide It is also known as niacinamide, Pyridine-3-carboxamide, nicotinic acid amide, or vitamin B_3 It is one of the two principle forms of B-complex vitamin, Niacin. It is a potent inhibitor of PARP. Nicotinamide has anti-diabetogenic [1], antioxidant[2], anti-inflammatory[3] and anticarcinogenic[4] activities. It has putative activity against osteoarthritis[5] and granuloma annulare[6].

Nicotinic acid It is Pyridine-3-carboxylic acid, a water soluble B-complex vitamin. It is used in the treatment of Atherosclerosis because it is a hypolipidemic agent and also used in the treatment of multiple sclerosis[10].The exact mechanism of action of nicotinic acid in multiple sclerosis is not known. Some evidences suggest that it dilates the blood vessels of the brain and provide more oxygen to the brain and reduce the symptoms [11].

Fampridine Several clinical trials have demonstrated that 4-amino pyridine, a potassium channel blocking agent, improves symptoms in some patients with multiple sclerosis. The beneficial effects have typically been attributed to the restoration of conduction to demyelinated neurons. Partition coefficient of fampridine is 0.76 [12,14].

Isoniazid In multiple sclerosis there is decrease in the level of GABA in cerebrospinal fluid. Isoniazid increases the level of GABA due to which tremors may be treated. Partition coefficient of isoniazid is 0.84 [13]

MATERIALS AND METHODS

Chemistry

The purity of all the synthesized compounds were checked by thin layer chromatography on silica gel G as stationary phase and different solvent systems as mobile phase using iodine vapors as detecting agent. Melting points were determined by the Tempo melting point determination apparatus in open capillary tubes and are uncorrected. Elemental analyses were carried out on Perkin Elmer 2400 CHN Elemental Analyser. Infrared spectra were recorded on Shimadzu 8000 FTIR Spectrophotometer in KBr phase. Proton NMR spectra were done on Bruker Avance II 400 NMR Spectrometer using tetramethyl silane as internal standard.

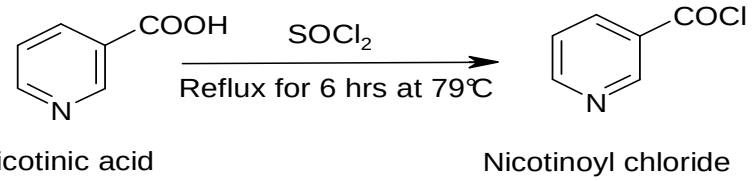
1.Synthesis of amide from Nicotinic acid and 4-amino pyridine (RS 01)

Step I - Synthesis of Nicotinoyl chloride 0.01mole of Nicotinic acid (1.23 gm) was refluxed with thionyl chloride (20 ml) for 6 hrs at 79°C on oil bath. The excess of solvent was evaporated under vacuum in rotary evaporator. Needle shaped, pale yellow crystals formed were collected.

Step II - Synthesis of N-(pyridine-3-carbonyl)-Isonicotinamide 0.01 mole of 4- amino pyridine (0.94 gm) was suspended in chloroform (15 ml) and added to a solution of nicotinoyl chloride in chloroform (15 ml) drop by drop at 10-15°C. 0.01 mole of anhydrous pyridine (2.5 ml) was added to the mixture drop wise. Then it was stirred for 25 hrs at room temperature on magnetic stirrer. Excess of solvent (chloroform) was evaporated under vacuum. The product was collected, dried and purified by column chromatography. The purity of the compound was ascertained by single spot T.L.C. using solvent system, Chloroform: Methanol (14:6), **R_f value**:0.37

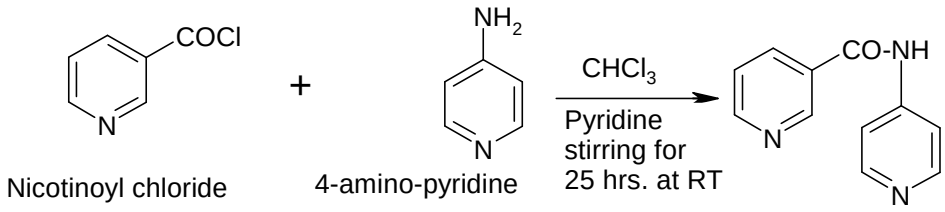
Figure-1

Step – I



Nicotinic acid Nicotinoyl chloride

Step-II



Nicotinoyl chloride 4-amino-pyridine Amide (1)

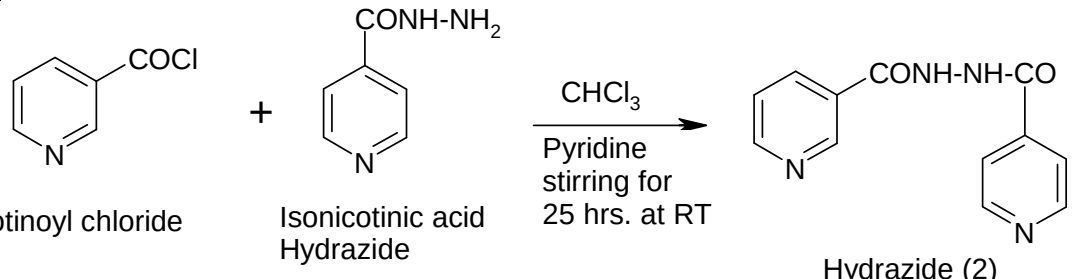
Melting point : 72-74°C, **Yield**= 83%. IR (KBr, cm^{-1}): 3320, 3119 N–H *str*, 3320-3010 C–H *str* (aromatic and hetero-aromatic), 1695,1641 C=O *str* (amide I for 2° amide), 1556 N–H *def* (amide II for 2° amide), 809 & 712 C–H *def* (aromatic and hetero-aromatic). ^{1}HNMR ($CDCl_3$): δ (ppm) 9.17-6.64 (m, 8H, Ar**H**), 8.0(s,1H,NH-sec amide) Anal.: Calculated (%) for $C_{11}H_9N_3O$: C,66.3; H,4.52; N,21.1,O,8.04. Found (%): C,66.4; H, 4.53; N, 21.3.,O,8.07

2.Synthesis of Hydrazide from nicotinic acid and isonicotinic acid hydrazide (RS 02)

Synthesis of Isonicotinic acid N-(pyridine-3-carbonyl)-hydrazide

0.01 mole of isonicotinic acid hydrazide was suspended in chloroform (15 ml) and added to a solution of nicotinoyl chloride (prepared as above) in chloroform (15 ml) drop wise at 10-15°C.0.01 mol of anhydrous pyridine (2.5 ml) was also added to the mixture drop wise. It was stirred for 25 hours on magnetic stirrer at room temperature. The excess of solvent was evaporated under vacuum in rotary evaporator. The product was collected, dried and purified by column chromatography. The purity of the compound was ascertained by single spot T.L.C. using solvent system, Chloroform: Methanol (14:6),**R_f value**: 0.68

Figure-2



Nicotinoyl chloride Isonicotinic acid Hydrazide Hydrazide (2)

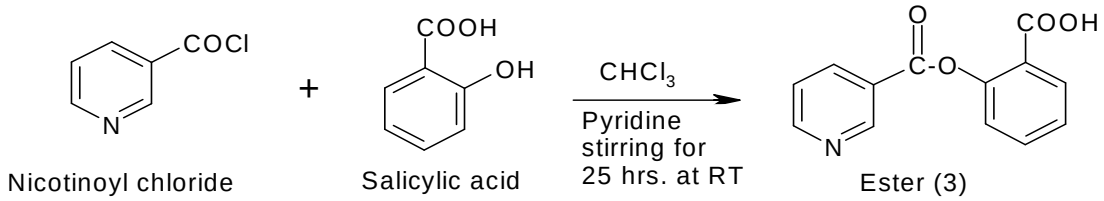
Melting point : 140-142°C **,Yield**: 85% IR (KBr, cm^{-1}): 3320, 3119 N–H *str*, 3320-3010 C–H *str* (aromatic and hetero-aromatic), 1695,1650 C=O *str* (amide I for 2° amide), 1557 N–H *def* (amide II for 2° amide), 809 & 712 C–H *def* (aromatic and hetero-aromatic). ^{1}HNMR ($CDCl_3$): δ (ppm) 9.17-7.63 (m, 8H, Ar**H**), 8.0(d,2H,NH-sec amide) Anal.: Calculated (%) for $C_{12}H_{10}N_4O_2$: C, 59.50; H,4.16; N,23.13,O,13.21. Found (%): C, 59.52; H,4.14; N,23.15,O,13.24.

3. Synthesis of ester from nicotinic acid and salicylic acid (RS 03)

Synthesis of N-(pyridine-3-carboxylate)-benzoic acid

0.01 mole of salicylic acid was dissolved in 15 ml of anhydrous pyridine. The solution was added to nicotinoyl chloride(as prepared above) in anhydrous pyridine drop by drop at 0°C. It was stirred at room temperature for 8 hrs on magnetic stirrer. The stirred mixture was poured into 200 ml of cold distilled water. Precipitates were formed and filtered off. Subsequent washings with cold water were done in order to remove pyridine from the product. The product was collected, dried and purified by column chromatography. The purity of the compound was ascertained by single spot T.L.C. using solvent system, Chloroform: Methanol(12:8),**R_f value**: 0.59

Figure-3



Melting point 78-80°C, **yield**: 81% IR (KBr, cm^{-1}): C-O-O stretching – 1701.1 cm^{-1}, -COOH stretching -1716.5 cm^{-1}, C-O-C symmetric stretching – 1147.5 cm^{-1}, C-O-C asymmetric stretching – 1261.4 cm^{-1}, =C-H- out of plane bending – 746.4 cm^{-1}, 894.9 cm^{-1}, Broad OH stretching–2700-3100 cm^{-1}, Aromatic ring stretching–1458.1 cm^{-1} ^{1}HNMR ($CDCl_3$): δ (ppm) 9.04-7.28 (m, 8H, Ar**H**), 11.0 (s, OH, COOH) Anal.: Calculated (%) for $C_{13}H_9NO_4$:C,64.20;H,3.73;N,5.76,O,26.31.Found(%):C,64.30;H,3.71; N,5.77,O,26.33.

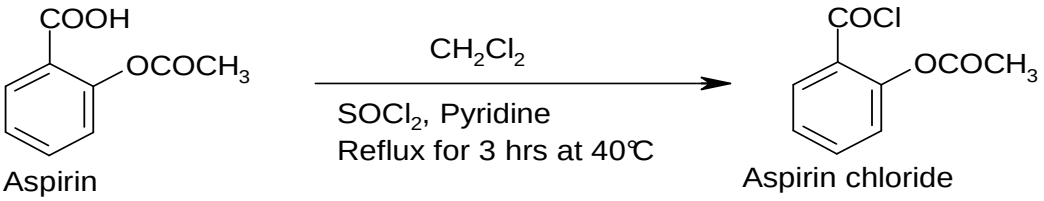
4.Synthesis of Imide from Nicotinamide and Aspirin (RS 04)

Step I - Synthesis of Aspirin chloride 8.31 mmoles of Aspirin (1.5 gm) was suspended in 6.3 ml of CH_2Cl_2 at room temperature, and then 0.249 mmoles of pyridine (0.02 ml) was added. Solution was cooled at 0°C. Then 9.15 mmoles of $SOCl_2$ (0.675 ml) was added to it. The mixture was refluxed for 3 hrs at 40°C on water bath. The excess of solvent was evaporated under vacuum. The product was collected.

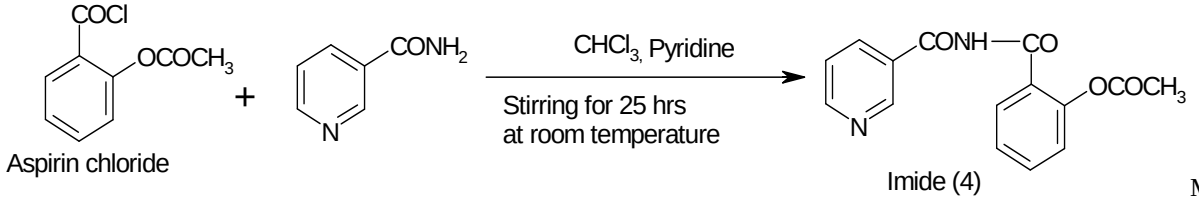
Step II -Synthesis of N-pyridine-3-(2-acetoxy benzoyl) carboxamide 8.31 mmol of nicotinamide (1.01 gm) was suspended in chloroform (15 ml) and added to solution of aspirin chloride in chloroform (15 ml), drop wise 10-15°C. 8.31 mmoles of anhydrous pyridine (2 ml) was also added to it drop wise. Then it was stirred for 25 hrs on magnetic stirrer at room temperature. Excess of solvent was evaporated under vacuum in rotary evaporator. The product was collected and dried in vacuum desiccator .The product was obtained as a dark brown extract. To this extract, methylene chloride was added for precipitation of product. The product was collected, dried and purified by column chromatography. The purity of the compound was ascertained by single spot T.L.C. using solvent system, Chloroform: Ethyl acetate (12:8),**R_f value**: 0.49

Figure-4

Step- I



Step-II



M

elting point 182-184°C, **Yield**: 79% IR (KBr, cm^{-1}):Aromatic CH stretch – 3320-3010 cm^{-1},C = O stretching – 1662.5 cm^{-1} NH stretching – 3342.4 cm^{-1}. C-O-C symmetric stretching – 1186.1 cm^{-1}, C-O-C asymmetric stretching – 1292.2 cm^{-1}, =C-H- out of plane bending – 717.5 cm^{-1}, 821.6 cm^{-1}, Aromatic ring stretching – 1346.2 cm^{-1} ^{1}HNMR ($CDCl_3$): δ (ppm) 9.17-7.25 (m, 8H, Ar**H**), 10.0 (s,1 H,NH),2.08 (s,3H,CH_3) Anal.: Calculated (%) for $C_{15}H_{12}N_2O_4$: C,63.38; H,4.25; N,9.85,O,22.51. Found (%): C,63.36; H,4.23; N,9.87,O,22.53.

Procedure for the determination of partition co-efficient

Partition coefficient was determined in chloroform and distilled water. Accurately weighed quantity of compounds (10 mg) were taken in glass stoppered tubes containing equal volumes (10 ml) of chloroform and distilled water. The tubes were shaken for six hrs using shaker water bath. The tubes were allowed to stand for 1 hr, so that the layers got separated. After that, aqueous phase was separated and absorbance was measured after making appropriate dilutions using UV spectrophotometer. Results are shown in the table.

Table 1 : Partition Coefficient of the Synthesized Compounds

Compounds	Abs.	Conc. (µg/ml)	mg of Drug in distilled water	mg of Drug in $CHCl_3$	Partition Coefficient $P = \frac{C(org)}{C(aq)}$
Nicotinic acid	0.684	62.2	6.2	3.8	0.61
Nicotinamide	0.801	65.3	6.5	3.5	0.54
RS 01	0.436	23.9	2.3	7.7	3.34
RS 02	0.273	25.9	2.5	7.5	3.00
RS 03	0.139	30.6	3.0	7.0	2.33
RS 04	0.094	46.3	4.6	5.4	1.17

RESULTS AND DISCUSSION

Twin Drugs of Nicotinamide and Nicotinic acid were synthesized The strategy for the synthesis of derivatives of Nicotinic acid involves formation of nicotinoyl chloride using thionyl chloride and then condensation of nicotinoyl chloride with respective hydrazide, acid and amine. Similarly synthesis of derivatives of nicotinamide involves formation of aspirin chloride using methylene chloride and then condensation of aspirin chloride with nicotinamide.Nicotinic acid has been combined with Fampridine and Isoniazid, all used in the treatment of multiple sclerosis. Nicotinic acid has been combined with Salicylic Acid, both used in the treatment of atherosclerosis. PARP inhibitor i.e. Nicotinamide has been combined with COX inhibitor i.e. Aspirin, having anti-inflammatory activity. These were characterized on the basis of their elemental and spectral analysis. Infrared spectra of each compound showed bands for N–H stretching vibrations at about 3320 cm–1 and 3315 cm–1 and C–H stretching vibrations for aromatic and hetero-aromatic moiety were observed in the range of 3080-3015 cm–1.

(C=O str) for secondary amide was observed near 1640 cm–1.Stretching vibrations (C=O str) for carboxylic acid moiety was observed near 1695 cm–1. Aromatic deformation vibrations were found near 805 and 715 cm–1. In case of 1HNMR spectra the chemical shift values for aromatic protons was found in the range of 8.26-7.01 δ (ppm). Aromatic methyl groups (Ar–o-CH3 and Ar–m-CH3) were found at 2.14 and 2.32 δ (ppm) as singlet respectively. The results of elemental analyses were found in good agreement with the calculated values. All the compounds were found to have greater value of partition coefficient than the parent drug, i.e. nicotinamide, nicotinic acid, fampridine and isoniazid.

CONCLUSION

Some new Twin Drugs of Nicotinamide and Nicotinic acid were prepared and the structure was established on the basis of FTIR & NMR Spectra. Synthesized compounds have greater partition coefficient than the parent drug so it may be concluded that the side effects and dosing may be reduced with increase in its bioavailability because of increase in lipophilicity.

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