Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

SYNTHESIS, CHARACTERIZATION AND ANTI-MICROBIAL ACTIVITY OF SOME NOVEL SUBSTITUTED N-[2-OXO-1-(ARYL/HETEROARYL-1-YLMETHYL)INDOLIN-3-YLIDENE]NICOTINOHYDRAZIDE DERIVATIVES

RAMALAKSHMI NATARAJAN[1], ARUNKUMAR SUBRAMANI[2], KARTHICK[1] SATHESH KUMAR KESAVAN[4] DEEPA SELVARAJ[3*]

[1]Department of Pharmaceutial Chemistry, C.L.Baid Metha college of pharmacy, Thooraipakkam, [2]Department of Pharmaceutical Chemistry, S.R.M. college of pharmacy, SRM university, Kattankulathur, [3]Department of Pharmaceutical Chemistry, [4]Department of Pharmaceutics, Faculty of pharmacy, Sri Ramachandra University, Porur, Chennai, India. Email: deepaselvarajs@gmail.com

Received: 17 Oct 2013, Revised and Accepted: 09 Nov 2013

ABSTRACT

In general, essential oils of spices acquire physically powerful anti-bacterial properties in opposition to food borne pathogens and contain high concentrations of phenolic compounds. These compounds show symbols of extensive variety of biological special effects, counting anti-microbial property.

Aim and Objective of the study: The belief of present study is to synthesis some nicotinic acid derivatives and evaluates their anti-microbial properties.

Methods: A series of novel Mannich containing N-[2-oxo-1-(Aryl/heteroaryl-1-ylmethyl)indolin-3-ylidene]nicotinohydrazide moiety has been synthesized II(a-k) characterized by the spectroscopic data and designed to calculate for their anti-microbial activity.

Result: The result of the anti-bacterial activity and anti-fungal activity evaluation of N-[2-oxo-1-(Aryl/heteroaryl-1-yl methyl) indolin-3-ylidene]nicotinohydrazide derivatives II (a-k) proved to be comparable or more potent with respect to the reference drugs. In particular compounds N-[1-((3-Chloropiperidine-1-yl)methyl)-2-oxoindoline-3-ylidene] nicotine hydrazine II-d, N-[1-((4-Nitrophenylamino)methyl)-2-oxoindoline-3-ylidene] nicotine hydrazide III exhibited potent anti-microbial and anti-fungal activity.

Conclusion: In the present study, a series of N-[2-ox0-1-(Aryl/heteroaryl-1ylmethyl) indolin-3-ylidene]nicotinohydrazide II(a-k) were synthesized with the belief of estimating its anti-microbial possessions. The title compounds possess reasonable to fine anti-microbial effectiveness.

Keywords: Anti-bacterial activity/ anti-fungal activity/ Mannich base/ Synthesis

INTRODUCTION

Formerly, a large quantity of nicotinic acid derivatives was equipped in our research lab for their anti-bacterial and anti-mycotic activities [1, 2]. In prolongation we planned to synthesis some Isatin mannich bases containing Nicotinic acid hydrazide. With the emergence and raise of microbial organisms dead set against to manifold anti-biotic, and the long-lasting emphasis on health – care costs, many researchers have tried to expand new, valuable anti-microbial reagents free of resistant and cost [3,4].

MATERIALS AND METHODS

Chemistry

The sequence of reactions engaged for building of novel Nicotinic acid derivatives is outline in Scheme – I. The N-(2-oxoindoline-3-ylidene) nicotine hydrazide I was prepared by mixture of nicotinic acid hydrazide, Isatin and glacial acetic acid in methanol was heated under reflux for 2 hours and re crystallized from DMF and ethanol further synthesis of title compound II a were prepared by treating compound I with DMF, aromatic, hetero aromatic amines and substituted form II(a-k).The purity of the compound is inveterate by TLC using silica gel G as stationary phase and suitable solvent system as mobile phase as well as by melting point determination. Structures of all the recently synthesized compounds were recognized by the spectral data such as IR, H^1-NMR, C^{13}-NMR, Mass and elemental analysis and evaluated for its anti-microbial potency.

Melting point of newly synthesized compounds was resolute by open capillary tube method and is uncorrected. IR spectra were recorded on ABB Bomen FT/IR Spectrometer MB104 with KBr pellet (ABB Bomen FT/IR, Faridabad, India). ^1H and ^{13}C NMR were recorded on Brucker AV 500 Instrument (300 MHz; Brucker, Mumbai, India). All chemical shifts were recorded in parts per million (δ) units relative to internal standard TMS and DMSO-d_6 as solvent. Mass spectra were recorded on a GC-MS QP 5000 Shimadzu (Shimadzu Analytical (India) Pvt. Ltd, Mumbai, India) Elemental analysis (C, H and N) were performed on Perkin Elmer 240 CHN analyzer (Perkin Elmer life sciences Inc., Boston, MA, USA). All chemicals used in synthesis was obtained from E. Merck (Darmstadt, FRG) and Aldrich (Milwaukee, USA).The transparency of the compounds were restricted with Merck pre coated TLC plates and spots were envisaged with ultraviolet light. N-(4-fluorobenzyl)-2-Nitro-4-chloroaniline [5] m.p: 86-88°C (m.p.lit:88°C), N-(4-fluorobenzyl)-2-amino -4-chloroaniline [5]m.p: 95°C (m.p.lit:95°C) were geared up according to the literature [6, 7]. ATCC strains of the bacteria and fungus were procured from the culture collection of Refik Saydam Health Institution of Health Ministry, Ankara, Turkey.

Synthesis of N-(2-oxoindoline – 3- ylidene) nicotinohydrazide (I)

A mixture of nicotinic acid hydrazide, Isatin and Glacial acetic acid in methanol is heated under reflux for 2 hours. The solid that separated on cooling was filtered, washed with water, dried and re crystallized from DMF (Di methyl formamide) – Ethanol. Yield : 78.62 %, M.P: 264-266 °C. IR (υ_{max}, /cm,KBr) cm^{-1}: 3050 (Ar-C-H), 3447 (N-H) and 1659 (C=O).

General procedure for synthesis of N-[2-oxo-1-(Aryl/heteroaryl-1-ylmethyl)indoline-3-ylidene]nicotinohydrazide (II)

A mixture of mannich bases I (0.01 mol) suspended in a minimum quantity of DMF, to this formaldehyde 1ml amines (0.01 mol) were added with forceful stirring. The solution was warmed on a water bath for 2 min and then left at room temperature over night. Solid compound thus attained were filtered and dried out and re crystallized from chloroform – pet ether (60-80°C) (1:1).

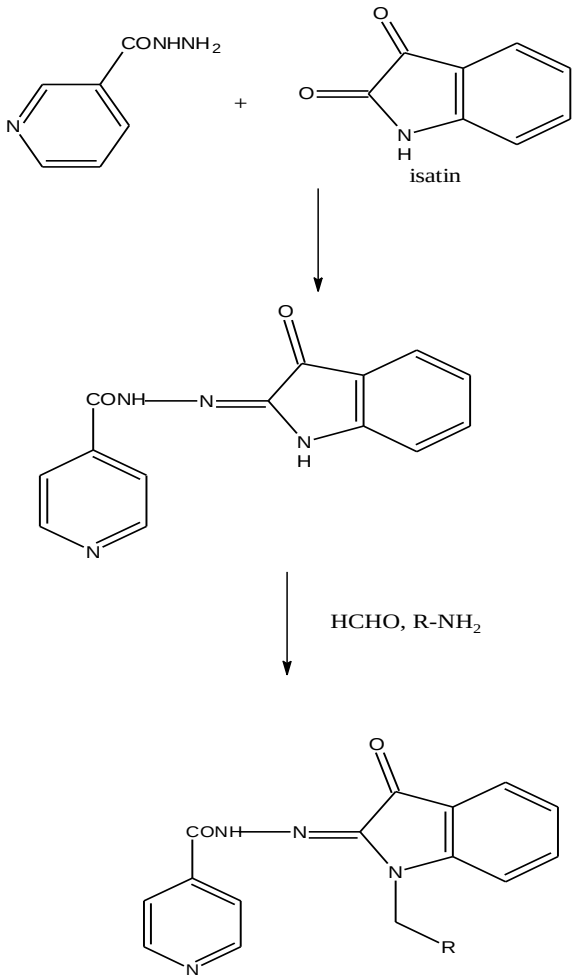
N-[2-oxo-1-(piperazine-1-ylmethyl)indoline-3-ylidene]nicotinohydrazide (IIa)

IR (υ_{max}, /cm,KBr): 3050 (Ar-C-H), 3447 (N-H) and 1659 (C=O); ^1H NMR (DMSO-d_6, 300 MHz) δ: 7.1 1H, s, N-H), 7.8-8.3 (8H, m,Ar-H)

p.p.m..^{13}C-NMR (DMSO-d_6) δ:46, 52.6,70.1,117.8,121.7,124.5,125.1, 129.4,130.7,131.3,132.8,147.2,153.7,163.0,163.5.p.p.m;

N-[2-oxo-1-(piperidine-1-ylmethyl)indoline-3-ylidene] nicotinohydrazide (IIb)

IR (υ_{max}, /cm,KBr): 3061 (Ar-C-H), 3239 (N-H) and 1672 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.4 (1H, s, N-H), 8.0-8.5 (8H, m, Ar-H).^{13}C-NMR(DMSO-d_6) δ:25.6,25.9,46,52.6,70.1,117.8,121.7,124.5, 125.1,129.4,130.7,131.3,132.8,147.2,153.7,163.0,163.5.p.p.m;



SCHEME – I

N-[2-oxo-1-((4-methylpiperazine-1-yl)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIc)

IR (υ_{max}, /cm,KBr): 3055 (Ar-C-H), 3405 (N-H) and 1650 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.2 (s,1H of N-H), 7.8-8.5 (m,8H,Ar-H).^{13}C-NMR(DMSO-d_6) δ:43.1,46,50.1,52.6,54.9,70.1,117.8,121.7, 124.5,125.1,129.4,130.7,131.3,132.8,147.2,153.7,163.0,163.5.p.p.m;

N-[2-oxo-1-((3-chloropiperazine-1-yl)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IId)

IR (υ_{max}, /cm,KBr): 3079 (Ar-C-H), 3379 (N-H) and 1727 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.3 (s,1H of N-H), 8.3-9.2 (m,8H,Ar-H).^{13}C-NMR(DMSO-d_6) δ:40.9,46,,52.662.9,69.6,70.1,74.4,117.8, 121.7,124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0, 163.5. p.p.m;

N-[2-oxo-1-(morpholinomethyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIe)

IR (υ_{max}, /cm,KBr): 3012 (Ar-C-H), 3374 (N-H) and 1728 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ:8.1(s,1H of N-H), 6.8-7.4 (m,8H,Ar-H).^{13}C-NMR(DMSO-d_6) δ:46,51.1,52.6,66.5,70.1,70.4,117.8,121.7, 124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0,163.5. p.p.m;

N-[1-((2-aminophenyl amino)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIf)

IR (υ_{max}, /cm,KBr): 3065 (Ar-C-H), 3365 (N-H) and 1649 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.1 (s,1H of N-H), 8.0-8.3 (m,12H,Ar-H).^{13}C-NMR(DMSO-d_6) δ: 46, 52.6,68.1,70.1,114.3, 117.8,121.7,124.5, 125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0,163.5. p.p.m;

N-[1-((2-methoxyphenyl amino)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIg)

IR (υ_{max}, /cm,KBr): 3055 (Ar-C-H), 3335 (N-H) and 1650 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.5 (s,1H of N-H), 6.6-6.9 (m,12H,Ar-H).^{13}C-NMR(DMSO-d_6) δ: 46, 52.6,68.4,70.1,114.3, 115.1,117.8,121.7, 124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0,163.5. p.p.m;

N-[1-((2-oxo-1-phenyl amino)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIh)

IR (υ_{max}, /cm,KBr): 3023 (Ar-C-H), 3323 (N-H) and 1675 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.4 (s,1H of N-H), 6.4-7.0 (m,12H,Ar-H).^{13}C-NMR(DMSO-d_6) δ: 46, 52.6,68.4,70.1,113.5,114.3,117.8, 121.7,124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0, 163.5. p.p.m;

N-[1-((p-toludino)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIi)

IR (υ_{max}, /cm,KBr): 3076 (Ar-C-H), 3378 (N-H) and 1654 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.5 (s,1H of N-H), 6.3-6.9 (m,12H,Ar-H).^{13}C-NMR(DMSO-d_6) δ: 24.3,46, 52.6,68.1,70.1, 114.3, 117.8,121.7, 124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0,163.5. p.p.m;

N-[1-((4-methoxyphenyl amino)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIj)

IR (υ_{max}, /cm,KBr): 3059 (Ar-C-H), 3339 (N-H) and 1650 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.7 (s,1H of N-H), 6.1-7.1 (m,12H,Ar-H).^{13}C-NMR(DMSO-d_6) δ: 46, 52.6,55.9,68.1,70.1, 114.3, 117.8, 121.7,124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0, 163.5. p.p.m;

N-[1-((4-hydroxyphenyl amino)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (IIk)

IR (υ_{max}, /cm,KBr): 3011 (Ar-C-H), 3376 (N-H) and 1727 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.1 (s,1H of N-H), 6.4-6.8 (m,12H,Ar-H).^{13}C-NMR(DMSO-d_6) δ: 46, 52.6, 68.1,70.1, 114.3, 117.8,121.7, 124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0,163.5. p.p.m;

N-[1-((3-nitrophenyl amino)methyl)-2-oxoindoline-3-ylidene] nicotinohydrazide (III)

IR (υ_{max}, /cm,KBr): 3047 (Ar-C-H), 3375 (N-H) and 1649 (C=O).^{1}H NMR(DMSO-d_6, 300 MHz) δ: 7.2 (s,1H of N-H), 6.5-6.8 (m,12H,Ar-H).^{13}C-NMR(DMSO-d_6) δ: 52.6, 68.1,70.1, 107.4,109.5,114.3,117.8, 121.7,124.5,125.1,129.4,130.7,131.3,132.8,147.4,148.2,153.7,163.0, 163.5. p.p.m;

Assay for *in-vitro* anti-microbial activity

Microbiology

The minimal inhibitory concentration (MIC) of the compounds was fired by the Dilution method.

Sample preparation

Each of the test compounds and standards (ciprofloxacin, ketoconazole) was dissolved in DMF, at concentration of 100 µg/ml. Further dilutions of the compounds and standards in test medium were prepared at the requisite quantities of 50,100 and 150µg/ml.

Culture of microorganism

The standard strains were obtained from the American Type Culture Collection (ATCC), Rockville, USA, and the pathological strains were procured from the Department of Microbiology, CEEAL ANALYTICAL

LAB, and Chennai, India. The anti-microbial activity of the synthesized compounds was screened against the reported bacteria and fungi.

Assay for in-vitro antimicrobial activity

MIC of the synthesized compound was determined by Agar streak dilution method [8.9]. A stock solution of the synthesized compound (100 µg Ml^{-1}) in Dimethyl Formamide was prepared and graded quantities of the test compounds were incorporated in the specified quantity of molten sterile agar (nutrient agar for antibacterial activity and Sabouraud dextrose agar medium for anti-fungal activity). Specified quantities of the medium (40-50°) containing the compound was poured into a petri dish to give a depth of 3-4mm and allowed to solidify. Suspension of the microorganism were prepared to enclose approximately 10^5 cfu mL^{-1} (Colony forming unit per milliliter) and applied to plates with serially diluted compounds in DMF to the tested and incubated at 37°C for 24hours and 48hours for bacteria and fungi respectively. The MIC was measured to be the lowest concentration of the test substance show signs of no visible growth of bacteria and fungi on the plate. The observed MIC is presented in Table no 1.

Table I: *In-vitro* antifungal and antibacterial activities results of Synthesized compounds II (a-k) and III (MIC: in µg/ml)

Compounds	Minimum inhibitory concentration (µg/mL)					
	Bacteria strains				Fungi strains	
	S.aureus	S.epidermids	K.pneumonia	P.aeruginosa	A.niger	A.fumigatus
IIa	33.6	35.2	35.6	48.2	36.3	36.2
IIb	6.5	35.9	37.4	39.2	4.5	35.4
IIc	41.2	40.1	43.6	45.2	46.7	38.6
IId	33.1	34.7	7.5	31.3	38.5	32.8
IIe	32.6	38.1	34.7	38.3	38.8	36.9
IIf	39.3	40.1	42.1	41.1	43.3	43.5
IIg	41.9	41.6	37.9	45.4	41.3	42.2
IIh	38.3	40.2	41.1	41.2	42.6	43.8
IIi	40.4	42.3	42.9	7.3	36.2	38.5
IIj	41.3	5.25	39.2	45.9	40.8	41.5
IIk	44.2	39.5	40.1	44.6	45.0	45.4
III	31.6	33.4	32.4	37.7	33.6	3.2
Ciprofloxacin (100 µg mL^{-1})	30	31	28	25	-	-
Fluconazole (100 µg mL^{-1})	-	-	-	-	30	29
Control	-	-	-	-	-	-

RESULTS AND DISCUSSION

All described, N-[2-ox0-1-(Aryl/heteroaryl-1ylmethyl) indolin-3-ylidene]nicotinohydrazide II(a-k) were evaluated in vitro for antibacterial activity against S. aureus (ATCC 25923), P.aeruginosa (ATCC1688), S.epidermids (ATCC 155), K.pneumonia (ATCC 11298), MRSA clinical isolate, and antifungal activity against A. niger (ATCC 1688) and A.fumigatus (ATCC 46645). DMSO as a control has no effect at 12.5% and 6.25% concentration against bacteria and fungi respectively. The results obtained specify that most of these compounds are more vigorous against fungi than bacteria. The MIC of the synthesized compounds was screened by Agar streak method. However, compounds IId and III displayed significant antibacterial activities against the specified strains which were near to Ciprofloxacin, in the control experiments (Table 1). Compound III exhibited the greatest antibacterial activity against both S. aureus and MRSA MIC value of 6.25 lg/mL. Compound IIb and III has the finest antifungal activity against A. niger similar to Fluconazole (MIC = 3.12 lg/mL). Compounds IIa, IIb, Iii, and III exhibited good antifungal activity (MIC = 3.12 lg/mL) against A.niger close to Fluconazole (MIC = 6.25 lg/mL). All synthesized compounds (IIa-l) exhibited moderate to good antibacterial and antifungal activity with an MIC range of 3.2-48.2 µg mL^{-1}. Compound IIb shows antibacterial activity against S.aureus(MIC:6.5 µg mL^{-1}) and antifungal activity against M.luteus (MIC:5.25 µg mL^{-1}). Compound shows antifungal activity against A.fumigatus (MIC: 3.2 µg mL^{-1}). Ciprofloxacin (100 µg mL^{-1}) and Fluconazole (100 µg mL^{-1}) was used as standard drugs.

CONCLUSION

In the present study, a series of N-[2-ox0-1-(Aryl/heteroaryl-1ylmethyl) indolin-3-ylidene]nicotinohydrazide II(a-k) were synthesized with the presumption of estimating its anti-microbial property. The title compounds possess moderate to good anti-microbial potency, However auxiliary *in vivo* studies is desired to wrap up any thing distinct about the therapeutic potential of these compounds.

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