RESEARCH ARTICLE ISSN: 2349-2678

Contents lists available at www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

NLM (National Library of Medicine ID: 101738825)

Index Copernicus Value 2017: 71.80

Volume 8 Issue 5; September-October; 2020; Page No. 01-04

SYNTHESIS, CHARACTERIZATION AND ANTI-MICROBIAL ACTIVITY OF 3-SUBSTITUTED 1*H*-INDOLE-2,3-DIONE ANALOGUES

Surya Naresh Chunduru*[1], Madhira Bhawanishankar[2], Rakesh Kumar[2]

[1]*Department of Pharmaceutical Sciences, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Churu Rd, Vidyanagari, Churela, Rajasthan 333001*

[2]*Shivam Pharmaceutical Studies and Research Center, Anand, Sojitra Rd, 388325 Gujarat*

Conflicts of Interest: Nil

Corresponding author: Surya Naresh Chunduru

ABSTRACT

Antimicrobial drugs have greatest contribution to therapeutics they are one of the few curative drugs. Antibiotics are the substances produced by microorganisms, which suppress the growth of or kill other microorganisms at very low concentrations. All chemicals were provided from our college. All solvents were redistilled before use. Reactions were routinely monitored by thin layer chromatography and spots were visualized by exposure to iodine vapour or UV light. All the synthesized compounds were purified by recrystallization. Melting points were determined by using open capillary method. and equimolar amines and 5-Nitro (R=NO_2) substituted isatins (1 mmol of each) were added to 96% w/w ethanol (20 mL) containing 8 drops of glacial acetic acid. The mixture was heated under reflux for 5 h and then cooled to room temperature. Antibacterial activities was test on nutrient medium against, *Staphylococcus aureus* (MTTC 4325), and *Escherchia coli* (MTTC 3265), which are representative types of gram positive and gram negative organisms respectively. The antibacterial activities of the compounds were assessed by disc-diffusion method. Compound IIIc and IIId shown excellent antibacteial against *E.coli* and *S.Aureus* bacteia, range from 20 to 24 nearly significant to standard drug amoxicilline, which shown 24 to 25mm on different two concentration i.e. 50 and 100 µg/ml, the substitution of phenol ring, pyridine ring at 3-substituted 1*H*-indole-2,3-dione analogues having responsible for significant antibacterial activity.

Keywords: 1*H*-indole-2,3-dione; Isatin; Antibacteial activity; Disc diffusion method.

INTRODUCTION

Microbiology is emerging as the key biological science. Microorganisms provide the models used in molecular biology for research. This research at the molecular level has provided, and continues to provide, the answers to numerous fundamental questions in genetics, metabolism, and cell forms and functions. Microorganisms also provide model systems for studying the relationships between species in mixed populations (1). The course of an infection is determined by three interacting factors: the microorganism, host resistance and treatment. The most important of these is the interaction between the host and the pathogenic microorganisms, i.e. the balance between the virulence of the pathogens and the resistance of the host to the pathogens. The role of antimicrobial agents, although often decisive, is mainly to shift the balance in favor of the host, giving the host time to metabolize its resistance mechanisms (2). Some bacterial species are naturally resistant to certain classes of antibiotics, either because they lack the necessary receptor or because their cell wall is impenetrable to the drug. There are several ways in which bacteria may acquire resistance. The most common mechanism of resistance is that the microorganisms acquire an enzyme that destroys the antibiotics (3). An important factor in the spread of resistance is the transfer of genetic material from one microorganism to another, even from a non-pathogen to a pathogen. Many pathogenic bacteria have developed resistance to the commonly used antibiotics (4). Antibiotics resistant in the bacteria spread at three levels: a) By transfer of bacteria between people, b) By transfer of resistant genes between bacteria (usually on plasmids) and c) By transfer of resistant genes between genetic elements within the bacteria, on transposons.

Tranposons: Some stretched of DNA can be fairly readily transferred (transposed) from one plasmid to another and also from plasmid to chromosomes or vice versa. This is because integration of these segments of DNA, which are called transposons (5).

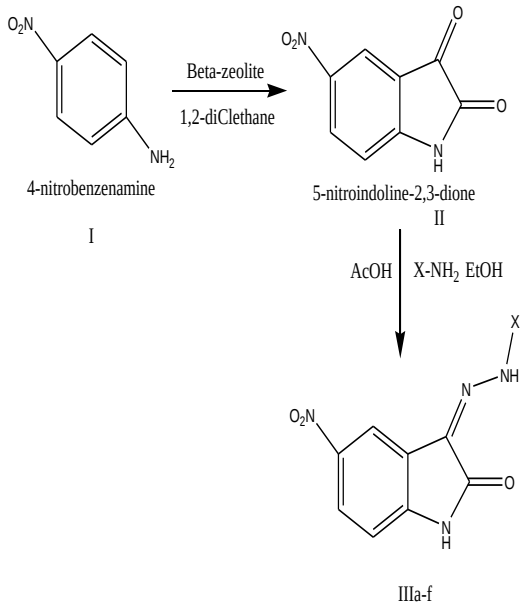
Antimicrobial drugs have greatest contribution to therapeutics. They are one of the few curative drugs. Antibiotics are the substances produced by microorganisms, which suppress the growth of or kill other microorganisms at very low concentrations (6). An antibiotic is said to have a narrow spectrum of activity, if it is effective against either Gram-positive or Gram-negative bacteria. Antimicrobial drugs can be classified in many ways according to their chemical structure, mechanism of action, types of organisms, spectrum of activity, type of action, source of origin. Antibiotics are bactericidal or bacteriostatic (7).

MATERIALS AND METHODS:

Chemical and reagents

All chemicals were provided from our college. All solvents were redistilled before use. Reactions were routinely monitored by thin layer chromatography and spots were visualized by exposure to iodine vapour or UV light. All the synthesized compounds were purified by recrystallization. Melting points were determined by using open capillary method.

Synthetic Scheme:



4-nitrobenzenamine

I

5-nitroindoline-2,3-dione

II

IIIa-f

X= a)Imidazolidine, b)carboxamide, c)carbothiomide, d)phenol, e)p-hydoxyphenol, f)pyridine

Synthetic Procedure:

The synthesis of 5-nitroindoline-2,3--dione starting from 4-nitrobenzenamine by using oxalyl chloride as the acylating agent and H-β zeoliteas a reusable catalyst in the presence 1,2-dichloroethane as solvent at 80 °C under heterogeneous conditions . The procedure requires simple filtration of the catalyst (8) and evaporation of the solvent to obtain good yields of 5-nitroindoline-2,3--dione 52.23%, and equimolar amines and 5-Nitro (R=NO_2) substituted isatins (1 mmol of each) were added to 96% w/w ethanol (20 mL) containing 8 drops of glacial acetic acid. The mixture was heated under reflux for 5 h and then cooled to room temperature. The resulting solid was collected by filtration, washed with cold ethanol and dried in open air. The derivatives thus prepared had sufficient analytical purity.(9)

Screening of antimicrobial evaluation:

Anti-bacterial activity:

All the compounds synthesize in the present investigation were screened for their anti-bacterial activity by Cup plate Method. Antibacterial activities was test on nutrient medium against, *Staphylococcus aureus* (MTTC 4325), and *Escherchia coli* (MTTC 3265), which are representative types of gram positive and gram negative organisms respectively. The antibacterial activities of the compounds were assessed by disc-diffusion method (10).

Procedure:

The sterilize media was cooled to 45^0C with gentle shaking to bring about uniform cooling and then inoculate with 18-24 hrs old culture under aseptic conditions, mixed well by gentle shaking. This was poured in to sterile Petri dishes (properly labelled) and allowed the medium to set. After solidification all the Petri dishes were transfer to laminar flow unit. Then the discs which were previously prepared were carefully kept on the solidified media by using sterilized forceps (11). These Petri dishes were kept as it is for one-hour diffusion at room temperature and then for incubation at 37^0C for 24 hours in an incubator. The extent diameter of inhibition after 24 hours was measure as the zone of inhibition in millimetres (mm) (12).

RESULT AND DISCUSSION:

All the synthesized compounds were light creamish to brown coloured crystalline solids. Most of the compounds are freely soluble in chloroform and other solvents like methanol, ethanol. The melting point of the compounds was in the range of 152ºC to 251ºC. IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr. All the synthesized compounds have shown characteristic stretching and bending in desired range. Mass spectra were obtained using. All the spectra were taken by direct infusion mass with ESI and APCI in positive and negative mode ionization ranging from 500-2000 m/e. All the compounds possess a molecular ion M^+, M+1 peak. The ^{1}H-NMR spectra of some of the compounds were studied in d6-DMSO on a Bruker II Avance 400 MHz NMR spectrometer. All the compounds show characteristic chemical shift from TMS in terms of δ ppm. δ value obtained in the desired range which signifies the presence of aromatic ring.

Table 1: Physiochemical data of synthesized compound

Compound	% yield	Rf value*	Mol. formula	Mol. Mass
IIIa	72.16	0.55	$C_{11}H_7N_5O_5$	289.20
IIIb	88.56	0.61	$C_9H_7N_5O_4$	249.18
IIIc	66.28	0.77	$C_9H_7N_5O_3S$	265.24
IIId	86.36	0.93	$C_{15}H_{10}N_4O_4$	310.26
IIIe	87.98	0.85	$C_{15}H_{10}N_4O_5$	326.26
IIIf	75.51	0.74	$C_{14}H_9N_5O_4$	311.25

*Solvent system for TLC Tolune: ethylacetate (7:3)

FT-IR Spectral Analysis:

The FTIR absorption values of 3-substituted 1*H*-indole-2,3-dione analogues have been recognised on the basis

of FTIR and were reported in the experimental section. All the synthesized derivatives exhibited identical peaks of N-H of hydrazine in the region of 3200-3300 cm^{-1}, this indicates the presence of the hydrazine moiety in all the synthesized compounds. All the compounds shows stretching in the ranges of 1640-1670 cm^{-1} and denotes the carbonyl group (C=O) present in the compounds. Apart from that common peak of C=N hydrazine stretching is observed in the region of 1400-1600 cm^{-1} of frequency. A strong band is observes in the region of 1640-1720 cm^{-1}shows the C=C presence in the compound.

Nuclear Magnetic Resonance studies:

IIIa:^{1}H NMR revealed that compound 1-[(Z)-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]imidazolidine-2,4-dione shows the characteristics peaks in benzene ring (A) of indole ring, imidazolidine ring (B) of benzene ring (C) attached to ring attached amino group (D) in the range of δ 7.0- 8.10 for 12 hydrogen. In ring E the peaks of amine group and nitro group was observed at δ 8.98 and δ 8.18 respectively.

IIIb:^{1}H NMR revealed that compound 1-[(Z)-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]carboxamide-2,4-dione shows the characteristics peaks in benzene ring (A) of indole ring, carboxamide ring (B) of benzene ring (C) attached to ring attached amino group (D) in the range of δ 6 – 8 for 10 hydrogen. In ring E the peaks of amine group and oxa group was observed at δ 8.13 and δ 8.11 respectively.

IIIc:^{1}H NMR revealed that compound 1-[(Z)-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]carbothiomide-2,4-dione shows the characteristics peaks in benzene ring (A) of indole ring, carbothiomide ring (B) of benzene ring (C) attached to ring attached amino group (D) in the range of δ 6.5- 8.20 for 14 hydrogen. In ring E the peaks of amine group and thio group was observed at δ 8.8 and δ 8.3 respectively.

IIId:^{1}H NMR revealed that compound 1-[(Z)-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]oxaphenyl-2,4-dione shows the characteristics peaks in benzene ring (A) of indole ring, phenol ring (B) of benzene ring (C) attached to ring attached amino group (D) in the range of δ 6.7- 8.3 for 8 hydrogen. In ring E the peaks of amine group and phenyl group was observed at δ 8.60 and δ 8.44 respectively.

IIIe: ^{1}H NMR revealed that 1-[(Z)-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]hydroxyphenyl-2,4-dione shows the characteristics peaks in benzene ring (A) of indole ring, phenol ring (B) of benzene ring (C) attached to ring attached amino group (D) in the range of δ 6.7- 8.50 for 12 hydrogen. In ring E the peaks of amine group and hydroxy group was observed at δ 10.2 and δ 8.5 respectively.

IIIf:^{1}H NMR revealed that 1-[(Z)-(5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]pyridine-2,4-dione shows the characteristics peaks in benzene ring (A) of indole ring, pyridine ring (B) of benzene ring (C) attached to ring attached amino group (D) in the range of δ 6.7-8.10 for 9 hydrogen. In ring E the peaks of amine group was observed at δ 9.9.

Mass Spectral Studies:

In IIIa compound, molecular ion peak should be at 289, but because of removal of chlorine group from the benzene ring, and complete removal of indole ring with imidazolidine group it is seen at 290 (M+1), in IIIb compound, molecular ion peak should be at 249.18, in IIIc compound, molecular ion peak should be at 265.24, but because of removal of thio substituted benzene ring, attached to isatin ring it is seen at 267.14 (M+2), in IIId compound, molecular ion peak should be at 310.26, but because of removal of oxo substituted benzene ring and attached to isatin ring, in IIIe compound, molecular ion peak should be at 326.26, In IIIf compound, molecular ion peak should be at 311.25.

Table 2: Anti-bacterial activity data of synthesized compounds:

Sr.No	compound	Concentration µg/ml	#*E.coli*	#*S.Aureus*
1	Amoxicillin	50	24	25
		100	25	25
2	IIIa	50	18	18
		100	19	19
3	IIIb	50	19	19
		100	**21**	**20**
4	IIIc	50	22	22
		100	**24**	**23**
5	IIId	50	19	19
		100	**20**	**20**
6	IIIe	50	18	18
		100	19	19
7	IIIf	50	17	16
		100	19	17

#Zone of inhibition of synthesized compounds: 6-8 mm poor activity, 9-12 mm moderate activity, 13-18 above good.

CONCLUSION:

Compound IIIc and IIId shown excellent antibacteial against *E.coli* and *S.Aureus* bacteia, range from 20 to 24 nearly significant to standard drug amoxicilline, which shown 24 to 25mm on different two concentration i.e. 50 and 100 µg/ml mention in table 2. Structure resemblence of above mention compounds (IIIc and IIId) isatin derivative having 5-nitro 1*H*-indole-2,3-dione with imdazolidine ring substituted with different fuctional

group i.e. phenol ring, pyridine isatin derivative having responsible for significant antibacterial activity.

REFERENCE:

1. Saad B, Azaizeh H, Abu-Hijleh G, Said O (2006). Safety of traditional Arab herbal medicine. Evidence Based Complementary and Alternative Medicine 3: 433-439.
2. Larrey D (1997). Hepatotoxicity of herbal remedies. Journal of Hepatology 26: 47-51.
3. Lau KM, He ZD, Dong H, Fung KP, But PP (2002). Anti-oxidative, anti-inflammatory and hepato-protective effects of Ligustrumrobustum. Journal of Ethnopharmacology 83: 63-71.
4. Rang HP, Dale MM, Ritter JM, Moore PK (2003). Pharmacology. 5th ed. Churchill Livingstone, London, pp. 217-212.
5. Sallustio BC, Holbrook FL (2001). In vivo perturbation of rat hepatocyte canalicular membrane function by diclofenac. Drug Metabolism and Disposition 29: 1535-1538.
6. Bohra, A. ;Bohra, S.P. and Bohra, A. (2002). Screening of antimicrobial activity of the Withaniasomnifera against five human Pathogenic bacteria. Current Agriculture. 26 : 131 - 133.
7. Caceres, A. ; Cabrera, O. ; Morales, O. ; Mollinedo, P. and Mendida, P. (1991). Preliminary screening of antimicrobial activity of Moringaoleifera. Journal of Ethanopharmacology. 33 : 213 - 216.
8. Offonry, S. U. and Achi, O. K. (1998). Microbial population associated with the retting of melon pods during seed recovery. Plant food hum. nutr. 52 : 37 - 47.
9. Nandkarni, A.K. ; Indian MateriaMedica. Volume I. 22nd Edn. Bombay popular prakashan.(1995).
10. Eloff, J. N. (1998). Which extractant should be used for the screening and isolation of antibacterial components from plants ? J. Ethnopharmacol. 60 : 1 - 8.
11. Wells BG, Schwinghammer TL, Malone PM, Kolesar JM, Rotschafer JC, Dipiro JT (2008). Pharmacotherapy Principles and Practice. McGraw - Hills Companies, Inc., pp. 487-488.
12. Buschmann H (2002). What is pain? In: Buschmann H, Christoph T, Friderichs E, Maul C, Sundermann B. Antimicrobial: From Chemistry and Pharmacology to Clinical Application. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.