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Design Synthesis and Antimicrobial Activity Studies of Some Novel Derivatives of Spirooxiindole

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ABSTRACT: There are alkaloids with complex structures that are sterically hindered and sometimes bearing contiguous stereogenic centers such as oxindoles. The oxindole is a heterocyclic aromatic organic compound constituted by the fusion of both benzene and pyrrolone rings. Oxindole functionalization can lead to the formation of a wide variety of derivatives. However, the presence of a methylene group at the third position (C-3) of the five-membered oxindole ring lead to a chiral tridimensional structure with a quaternary asymmetric center such as spirocyclopropyl oxindole, pyrrolidinyl spirooxindole, spirocyclohexyl oxindole and 3,3´-disubstituted oxindole derivatives

IndexTerms **– spirocyclopropyl oxindole, pyrrolidinyl spirooxindole, spirocyclohexyl oxindole**

INTRODUCTION

Spirooxindole alkaloids, as a family member of oxindole natural products in nature, were first isolated from Rubiaceae and Apocynaceae plants. The privileged scaffold of spirooxindole contains two basic substructure units: one is multiple functionalized oxindole, which can be used as hydrogen bond donors and acceptors to interact with biological targets. The other is a cycloalkyl or heterocyclic moiety fused at the C-3 position of oxindole. It provides an opportunity to regulate the liposolubility and other physicochemical properties of spirooxindole. Accordingly, the unique spatial architecture and significant biological activities of spirooxindole have long captured the great attention of researchers. Pharmacological effects described over the past decades for spirooxindole-based natural products and the modified derivatives are reported and include evaluations of anticancer, antimicrobial, anti–inflammatory, analgesic, anti- oxidant, antimalarial, antiviral, antiatherosclerotic, antidiabetic, and insecticidal activities. Some reports on the antagonistic/inhibitory action on cholinesterase, DNA cleavage, prolyl hydroxylase 2 (PHD2), mineralocorticoid receptor, and progesterone receptor.

NEED OF THE STUDY

In medicinal chemistry, natural products are considered as an effective way to obtain lead compounds with prominent bio- logical properties, which plays an essential role in the discovery process of numerous marketed therapeutic drugs. Recently, a multitude of excellent reviews have systematically introduced the importance of compounds derived from natural sources for treating diseases in humans, such as cancer, bacterial/virus infection, and mental disorders [4–6]. Spirooxindole-based alkaloids, as an important member of natural product families, have aroused tremendous attention due to their specific chemical structures and pharmacological properties. Structure of chiral spirooxindole is found in a wide range of natural products that usually have important biological activities for instance, anticancer, antimicrobial and antifungal, anti-HIV and antimalarial among others.

RESEARCH ENVISAGED

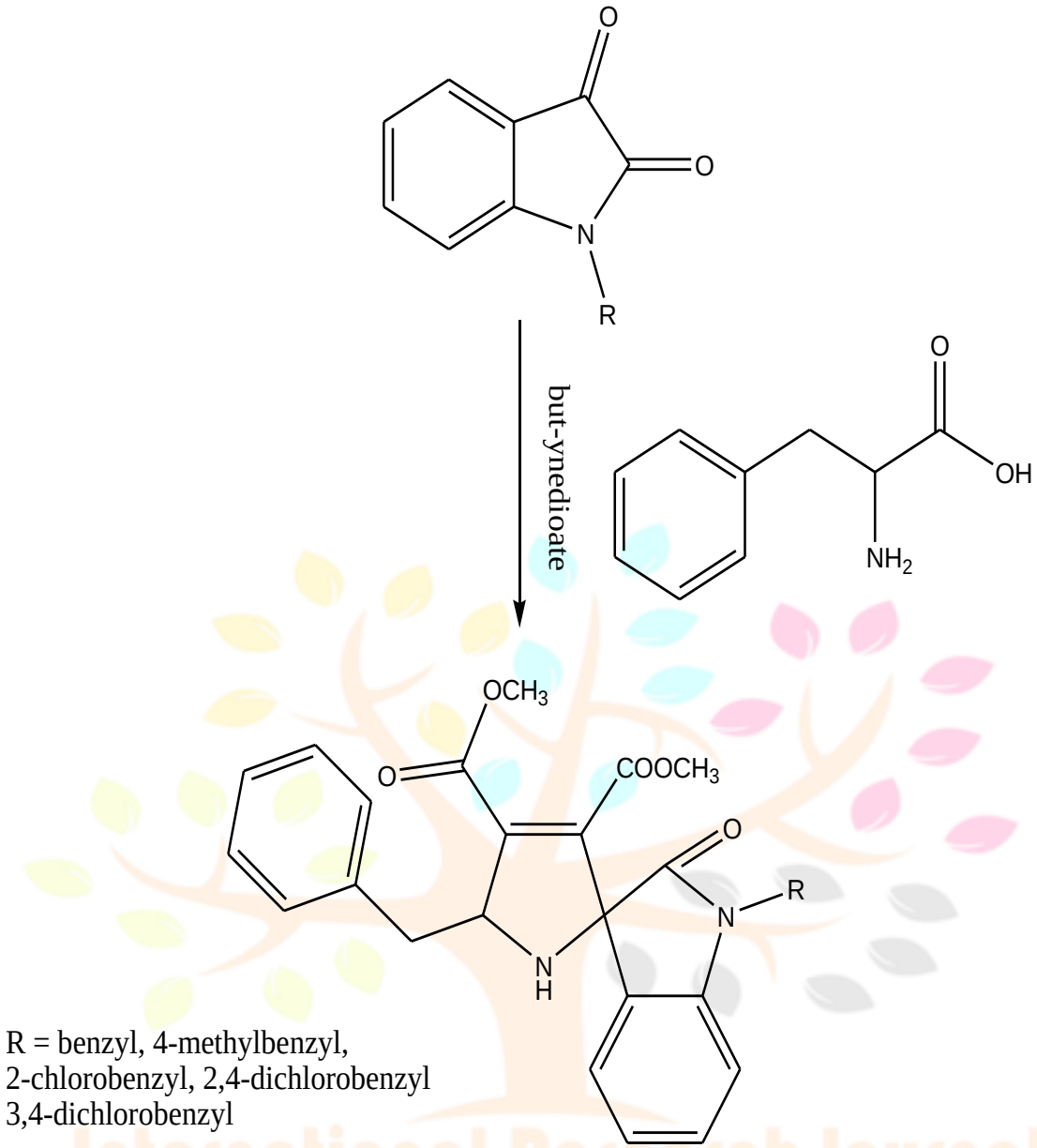
Although, number of molecules have been developed as antimicrobial agents. But, the demand for the effective and potent antimicrobial agent is always on high priority due to the development of resistant for the current drugs. Indole moiety is part of various natural products and medicinal agents. In literature, it has been reported that sharing of the indole-3 carbon atom during formation of spiroindoline derivatives greatly enhances its biological activity. Thus, considering this fact, we planned to synthesize some spiroxindole derivatives to improve antimicrobial efficacy.

The objective of the present investigation was to

1. Synthesize a few novel spirooxindole derivatives
2. Screen the spirooxindole derivatives for antimicrobial action against gram positive and gram negative bacteria

RESEARCH METHODOLOGY

Materials And Methods: All the material used for the present investigation have been procured from various sources and used as they were obtained.



Preparation of substituted Isatins

Anhydrous K_2CO_3 was added to a solution of the isatin (1mmol) and aryl halide (1mmol) dissolved in anhydrous DMF in a round bottom flask and was stirred at room temperature for 1-2 hr. After the completion of reaction checked by TLC, crushed ice was added into reaction mixture to precipitate out the crude product. The solid was filtered and washed with cold water.

Preparation of spirooxindoles

Isatin (1.0 mmol), ynedioate (1.0 mmol) and phenyl alanine (1.2 mmol) were dissolved in 15 mL of water and refluxed at 100°C for a period of 5 hours. The reaction mixture was diluted with 100 mL water and extracted with 50 mL ethylacetate. The organic layer was washed with saturated sodium chloride solution and dried over anhydrous sodium sulphate. The solvent was removed under vacuum evaporation to obtain the product.

Chemical Characterization

All the synthesized compounds were characterized for melting point, solubility, yield and elucidation of the structure. The structure elucidation was performed by spectroscopic analysis (NMR, Mass and IR).

Melting point

The melting points were determined by open capillary method and are uncorrected using a electrically heated melting point determination apparatus.

Thin Layer Chromatography

The purity and homogeneity of the compounds was determined by thin layer chromatography, using silica gel G as the stationary phase on glass plates. Iodine vapors were used for development of the chromatogram. The solvent system used for performing the TLC of compounds was hexane: methanol in the ratio 7:3.

Solubility

The solubility of all the synthesized compounds was qualitatively determined in different solvents. A small amount of the sample was shaken in 1 mL of solvent in a test tube and was visually inspected for the absence of the solid particles in the test tube.

Anitmicrobial Action

The antibacterial action of the synthesized compounds (obtained by microwave synthesis) was evaluated against two gram positive (*Proteus mirabilis* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*).

Preparation of test solutions

The synthesized spirooxindole derivatives were dissolved in dimethyl sulfoxide (DMSO) and the further dilutions of the test compounds were prepared at the required quantities of 100, 50, 25, 12.5 and 6.25 µg/mL concentrations with Mueller-Hinton broth medium.

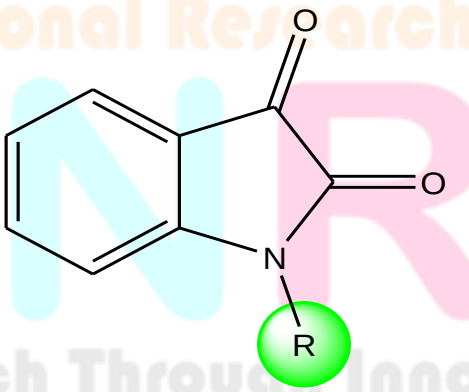
Preparation of Inoculum

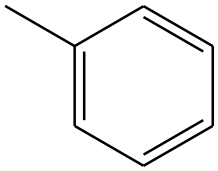
Overnight culture of all four bacteria were prepared separately in nutrient broth, and used as a microbial source for the determination of MIC.

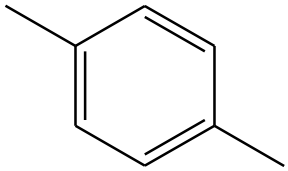
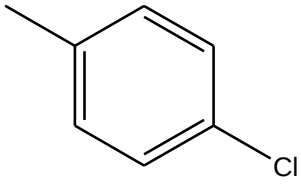
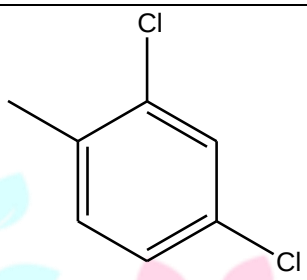
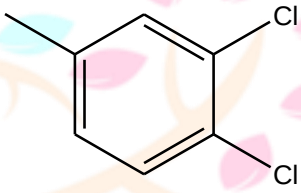
Determination of MIC

1. The sterile capped test tubes were numbered from 1 to 8 and all of the steps were carried out using aseptic technique.
2. 10 ml of drug sample solution was added to the first tube while 2.0 ml of nutrient broth to all other tubes.
3. Transferred 2.0 ml from the first tube to the second tube.
4. Using a separate pipette, mixed the contents of this tube and transferred 2.0 ml to the third tube.
5. Continue dilutions in this manner to tube number 8, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette.
6. Removed 2.0 ml from tube 8 and discard it. The 9th tube, which serves as a control, receives no drug sample.
7. Norfloxacin (10µg/ml) was used as standard drug.
8. Suspended to an appropriate turbidity several colonies of the culture to be tested in 5.0 ml of nutrient broth to give a slightly turbid suspension.
9. Added 0.2 µl of the diluted culture suspension to each of the tubes. The final concentration of drug sample is now one-half of the original concentration in each tube.
10. Incubated all tubes at 37°C overnight.
11. Examined tubes for visible signs of bacterial growth. The highest dilution without growth is the minimal inhibitory concentration (MIC).

List of substitution used for preparing various isatin



Compound	R
1a) Benzyl	

1b) 4-methylbenzyl	
1c) 2-chlorobenzyl	
1d) 2,4-dichlorobenzyl	
1e) 3,4-dichlobenzyl	

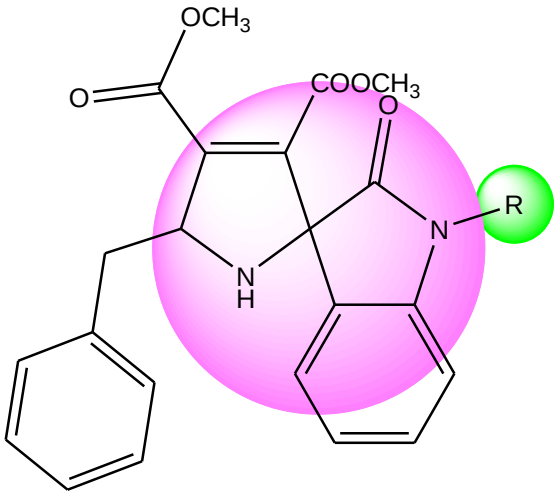
Physicochemical features of spirooxindole-3,2'-pyrrolidine compounds 4a-e

Compound	Color	Yield (%)	Melting Point (°C)
4a	White	74	239-240
4b	White	71	234-236
4c	Pale White	65	261-263
4d	Pale Yellow	61	278-281
4e	Pale Yellow	61	280-282

Solubility of compounds 4a-e

Compound	Water	Methanol	Chloroform	DMSO
4a	Insoluble	Poorly soluble	Poorly soluble	Soluble
4b	Poorly soluble	Poorly soluble	Poorly soluble	Soluble
4c	Soluble	Soluble	Soluble	Soluble
4d	Poorly Soluble	Poorly Soluble	Poorly Soluble	Soluble
4e	Poorly Soluble	Poorly Soluble	Poorly Soluble	Soluble

Structure of compounds was determined using proton NMR in DMSO-d6 solvent, IR and Mass study.



Compound 4a Molecular Formula: $C_{29}H_{26}N_2O_5$
FT-IR (cm^{-1}): 3342 (N-H), 3907 (C-H aliphatic), 1690.78 (C=O)
1H-NMR (DMSO-d6) (δ ppm): 2.087 (N-H), 2.541 (CH methylene), 3.881 (CH methoxy), 6.962-7.980 (CH Aromatic)
Mass (m/e): 484.9 (M^++2)

Compound 4b Molecular Formula: $C_{30}H_{28}N_2O_5$
FT-IR (cm^{-1}): 2925 (C-H aliphatic), 1741 & 1683.50 (C=O)
1H-NMR (DMSO-d6) (δ ppm): 2.087 (N-H), 2.750-2.541 (CH methylene), 3.881 (CH methoxy), 6.962-7.980 (CH Aromatic)
Mass (m/e): 497.2 (M^+)

Compound 4c Molecular Formula: $C_{29}H_{25}ClN_2O_5$
FT-IR (cm^{-1}): 3340.46 (N-H), 2756.18 (C-H aliphatic), 1699.89 (C=O)
1H-NMR (DMSO-d6) (δ ppm): 2.082 (N-H), 2.539 (CH methylene), 3.884 (CH methoxy), 6.952-7.983 (CH Aromatic)
Mass (m/e): 517.0 (M^+)

Compound 4d Molecular Formula: $C_{29}H_{24}Cl_2N_2O_5$
FT-IR (cm^{-1}): 2759.22 (C-H aliphatic), 1741.65 & 1682.94 (C=O)
1H-NMR (DMSO-d6) (δ ppm): 2.085 (N-H), 2.576 (CH methylene), 3.980 (CH methoxy), 6.807-8.018 (CH Aromatic)
Mass (m/e): 550.1 (M^+)

Compound 4e Molecular Formula: $C_{29}H_{24}Cl_2N_2O_5$
FT-IR (cm^{-1}): 2756.14 (C-H aliphatic), 1748.01 & 1690.93 (C=O)
1H-NMR (DMSO-d6) (δ ppm): 2.085 (N-H), 2.576 (CH methylene), 3.980 (CH methoxy), 6.807-8.018 (CH Aromatic)
Mass (m/e): 550.1 (M^+)

Antibacterial Activity

The antibacterial activity of the synthesized compounds was evaluated at various concentrations to determine the MIC of each compound (Table 5.4 & 5.5).

MIC of the synthesized compounds against gram positive bacteria

Compound	MIC (µg/mL)	
	B. subtilis	***P. mirabilis***
4a	100	100
4b	100	100
4c	50	50
4d	50	50
4e	50	50
Norfloxacin	6.25	6.25

MIC of the synthesized compounds against gram negative bacteria

Compound	MIC (µg/mL)	
	P. aeruginosa	***E.coli***
4a	50	50
4b	50	50
4c	25	25
4d	12.5	12.5
4e	12.5	12.5
Norfloxacin	6.25	6.25

Discussion

The synthesis of the spirooxindole-3,2'-pyrrolidine compounds was done via the 1,3-cycloaddition of but-ynedioate. The generation of imine by the reaction of isatin with phenylalanine (amino acid) offers a readily reacting 1,3-dipolar intermediate for the cycloaddition reaction. Water was used as the solvent to offer a green method of synthesis. Previous study has shown that microwave assisted synthesis of spirooxindole-3,2'-pyrrolidine using water as solvent resulted in 89% yield of the compound.[25]

In the IR spectrum, the sharp absorption bands at 3340 corresponding to NH stretching and 1740 to 1680 corresponding to carbonyl groups were prominent along the fingerprint bands of the aromatic system. The NMR spectra revealed protons of amine, methoxy, phenyl as well as methylene groups. The presence of either the molecular ion peak of the isotopic peak was found in the mass spectra of the compounds confirming the formation of the compounds.

The antibacterial activity was determined using the broth dilution method to obtain the minimum inhibitory concentration (IC_{50}) of the synthesized compounds against two gram negative and two gram positive bacteria. The results revealed that the compounds were inactive against the gram positive bacteria, displaying an MIC of 100 µg/mL in comparison to the reference drug (6.25 µg/mL). The activity against the gram negative bacteria on the other hand was quite significant and was found to be affected by the substitution present. The presence of two chloro groups in the molecules led to the most potent compounds **4d** & **4e** with MIC value of 12.5 µg/mL each. The position of the chloro substitution did not significantly affect the activity of the molecules against any of the tested bacteria.

SUMMARY AND CONCLUSION

The objective of this investigation was to synthesize spirooxiindole derivatives and evaluate them for antimicrobial action.

Summary

The synthesis was carried out in two steps involving derivatization of isatins and subsequent synthesis of spirooxiindoles using the substituted isatins. The synthesis of the spirooxindole-3,2'-pyrrolidine compounds was done via the 1,3-cycloaddition of but-ynedioate. Five N-substituted isatin were utilized as the starting material to obtain five distinct derivatives of spirooxindole-3,2'-pyrrolidine The compounds were obtained in yield of 61-74% and displayed varying solubility with all compounds soluble in DMSO.

The structure of the compounds was determined using proton NMR in DMSO-d6 solvent, IR and Mass study.

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Conclusion

In the present study, spirooxindole-3,2'-pyrrolidine compounds were synthesized using the reaction of isatin and phenylalanine to prepare the reactive intermediate. The compounds were found to be of good purity and yield. The compounds exhibited good antibacterial potential against gram negative bacterium tested. Two compounds **4d** & **4e** were found to be significantly potent. These compounds would be used for designing of newer spirooxindole-3,2'-pyrrolidine compounds with the aid of computer aided drug design techniques like pharmacophore modeling or docking which might lead to generation of a new lead molecule for antibacterial activity even against the multidrug resistant strains.

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