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Synthesis, characterization and biological activity of novel spiroheterocycles from isatin derivatives

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ABSTRACT

*Reaction of isatin with acetophenone derivatives gave 3-hydroxy-3-phenacyl oxindole derivative (**6**), dehydration of (**6**) gave 3-phenacylidene-2-indolinone derivative (**7**). Reaction of (**7**) with sarcosine and acenaphthenequinone yield novel spirooxindole derivatives which are characterized by various spectral methods. The compounds were subjected to antibacterial and antifungal studies and the results are very promising.*

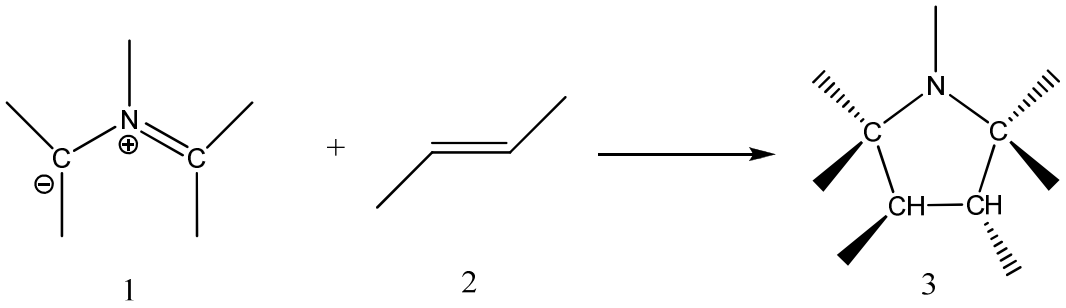
Keywords: 1,3-Dipolar cycloaddition, Azomethine ylides, oxindole, spirooxindole, Antimicrobial activity.

INTRODUCTION

The biological and pharmacological properties of isatin and its derivatives have led to extensive use of these compounds as key intermediates in organic synthesis[1]. Isatin is a core constituent of many alkaloids[2] and drugs[3] as well as dyes[4], pesticides and analytical reagents. Literature surveys reveal that various derivatives of isatin possess diverse activities such as antibacterial[5], antifungal[6],antiviral[7],anti-HIV[8],anti-mycobacterial[9],anticancer[10],antiinflammatory[11] and anticonvulsant activities[12].

The 1,3-dipolar cycloaddition, also known as the Huisgen cycloaddition or Huisgen reaction, is an organic chemical reaction belonging to the larger class of concerted, pericyclic cycloadditions. It is the reaction between a 1, 3-dipole and a dipolarophile, most of which are substituted alkenes, to form a five-membered ring. Rolf Huisgen first saw the prospects of varying the 1, 3-dipole and its high value for synthesis of 5-membered heterocycles[13]. The addition of a 1, 3-dipole to an alkene for the synthesis of five-membered rings is a classic reaction in organic chemistry. The 1, 3-dipolar cycloaddition reactions are used for the preparation of molecules of fundamental importance for both academia and industry. The history of 1, 3-dipoles goes back to Curtius, who in 1883 discovered diazoacetic ester[14].

The 1,3-dipolar cycloaddition reaction of azomethine ylides**(1)** with alkenes**(2)** leads to the formation of the pyrrolidines**(3)**. 1, 3-Dipolar cycloaddition reaction of azomethine ylides forms highly substituted heterocyclic compounds.



SCHEME 1

Azomethine ylides are unstable species which have to be prepared in situ. A number of methods have been developed for the generation of azomethine ylides, such as proton abstraction from imine derivatives of R-amino acids, thermolysis or photolysis of aziridines, and dehydrohalogenation of imonium salts. The reaction of azomethine ylides with alkenes has been investigated from a theoretical point of view inorder to understand the reaction course, selectivity, and influence of Lewis acids on the reaction. The product can be used as new catalyst or serve as important biologically active molecules [15].

Spiro-cyclic compounds have attracted the attention of organic chemists due to their unique structural and reactivity pattern [16]. 1, 3-dipolar cycloaddtion offers a convenient route for the construction of five membered heterocyclic compounds. The 1, 3-dipolar cycloaddition reaction has been applied to the synthesis of natural products such as sugar derivatives, β-lactams, aminoacids and alkaloids. Isooxazoline derivatives have been extended to many natural product synthesis and also proved to be an efficient precursor for many synthetic intermediates including γ-amino alcohols, β-hydroxy ketones etc. The high synthetic versatility and the pharmacological importance have prompted to synthesize some biologically interesting spiroisocazoline derivatives. Manikandan and his coworkers done their work in the construction of novel spiroheterocyclic derivatives, and also to study their biological applications, the reactions of the versatile 1, 3-dipole nitrile oxide with 9-arylidene anthrone have been studied[16].

S.Kathiravan and his coworkers reported (E)5H-2-(arylidine)-5-phenyl-6,7-di-hydrothiazolo[2,3-b]benzo[h]quinazolines through a regioselective 1,3-dipolar cycloaddition reaction with azomethine ylide derived from ninhydrin and sarcosine to give a new class of complex dispiropyrrolidines in good yield. The structures of the synthesized cycloadducts have been elucidated by spectral methods[17].

T. Augustine and his coworkers reported the cycloaddition reaction of azomethine ylides, generated through decarboxylation, with (E)-3-arylidene-4-chromanones and Chalcones as dipolarophiles. A high degree of regioselectivity has been observed in the synthesis of a new class of functionalized dispiroheterocyclic compounds bearing chromanone, chalcones and acenaphthenequinone framework[18,19]. Spiro-oxindole ring system represents an important class of naturally occurring substances characterised by highly pronounced biological properties[20,21]. Oxindole derivatives are found to be potent aldose reductase inhibitors(ARI) , which help to treat and prevent diabetic complications arising from elevated levels of sorbitol[22-24].

As a part of our ongoing research program in the area of cycloaddition reaction of azomethine ylides with oxindole derivatives from isatin, we herein report the highly regioselective synthesis of spiro oxindoles through 1,3–dipolar cycloaddition methodology. The prepared compounds were characterized by spectroscopic techniques and its biological applications like antibacterial and antifungal studies were carried out.

MATERIALS AND METHODS

All chemicals used were of analytical grade, supplied from Sigma-Aldrich and used as received. The first step is reaction of isatin with acetophenone derivatives which was carried out in the presence of diethyl amine as a basic catalyst giving rise to 3-hydroxy-3-phenacyl oxindoles in good yields. Dehydration of the above compounds by dilute alcoholic hydrochloric acid gave 3-phenacylidene-2-indolinones in quantitative yields. Further,1,3 dipolar addition was carried out by generating azomethine ylides using acenapthenequinone and sarcosine that led to novel

spirooxindole systems. The structures of these products were established by physical and spectral methods. Melting points were measured on Gallen-Kamp apparatus**.**

STEP I

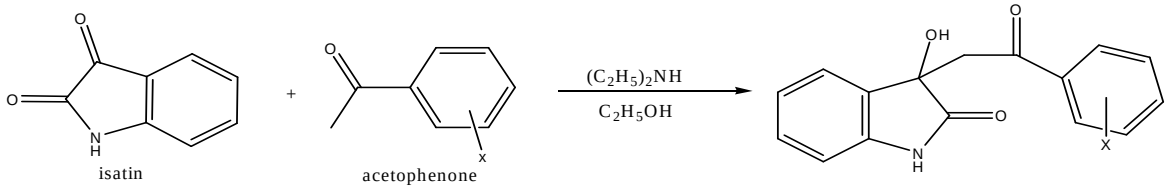
Preparation of 3-hydroxy-3-phenacyl oxindoles

A mixture of isatin and substituted acetophenone (0.01 mole of each) was dissolved in ethanol (100 mL) and diethyl amine (1 mL) was added. The mixture was allowed to stand overnight at room temperature, the yellow needles formed were recrystallised from ethanol.(**SCHEME 2**)

STEP II

Preparation of 3-phenacylidene -2-indolinones

A mixture of 0.01 mole of compound 6, ethanol 25 mL and 50 mL of dilute HCl solution (25%), was allowed to stand overnight, fine orange needles were formed. (**SCHEME 3**)



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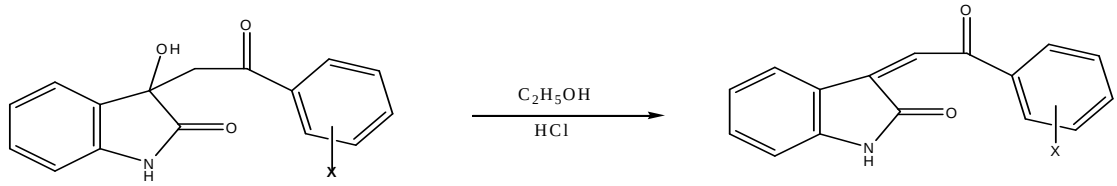
SCHEME 2

6



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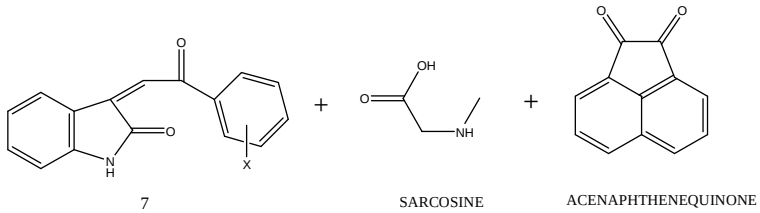
SCHEME 3



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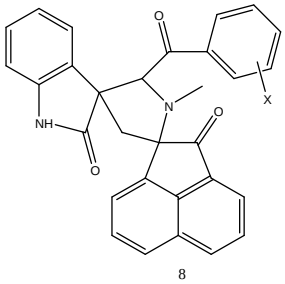

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X = H,p-Cl,p-OCH_3,m-NO_2 ,m-OH

SCHEME 4

STEP III

Preparation of spirooxindolinones

A mixture of compound II (0.01 mmole), acenaphthenequinone (0.01 mol) and sarcosine (0.01 mol) in ethanol(40 ml) was refluxed for 4 hours at a temperature of 60˚C. After the reaction was over, the reaction mixture was poured in crushed ice and the precipitate obtained was filtered and dried; then it was recrystallised using acetone. The melting point and chemical yield are 136˚C -140˚C and 87.7%. (**SCHEME 4**)

RESULTS AND DISCUSSION

***Spiro compound 8a(X=H)*:** IR (KBr): 1601, 1719 cm^{-1}; ^{1}H NMR (DMSO/400 MHz): δ 2.27 (s,3H), δ 4.12 (d, 1H), δ 4.01 (t, -NH), δ 6.3-7.7 (m, aromatic), δ 3.17 (s-2H); ^{13}C-NMR (DMSO/400 MHz): δ 77.81,151,162,195.13,201.9; m/s:458.83

Spiro compound 8b(X=p-Cl): IR (KBr): 1610, 1723 cm^{-1}; ^{1}H NMR (DMSO/400 MHz): δ 2.25(s,3H), δ 4.10 (d, 1H), δ 4.0 (t, -NH), δ 6.3-7.7 (m, aromatic), δ 3.17 (s-2H); ^{13}C-NMR (DMSO/400 MHz): δ 74.41, 132, 138.7,162,195.13,201.7; m/s:493.33

Spiro compound 8c(X=p-OCH_3): IR (KBr): 1610, 1723 cm^{-1}; ^{1}H NMR (DMSO/400 MHz): δ 2.25(s,3H), δ 4.10 (d,1H), δ 4.0 (t, -NH), δ 6.3-7.7 (m, aromatic), δ 3.17 (s-2H), δ3.73 (s,3H); ^{13}C-NMR (DMSO/400 MHz): δ 55.9, 74.41, 132,165.1,195.13,201.7; m/s:490.11

Spiro compound 8d(X=m-NO_2): IR (KBr): 1610, 1723 cm^{-1}; ^{1}H NMR (DMSO/400 MHz): δ 2.27(s,3H), δ 4.12 (d,1H), δ 4.0 (t, -NH), δ 6.35-8.82 (m, aromatic), δ 3.27 (s-2H); ^{13}C-NMR (DMSO/400 MHz): δ 74.41, 132, 148.3,195.13,201.7; m/s:504.83

Spiro compound 8e(X=m-OH): IR (KBr): 1610, 1723 cm^{-1}; ^{1}H NMR (DMSO/400 MHz):δ 2.27(s,3H), δ 4.12 (d,1H), δ 4.0 (t, -NH), δ 6.35-8.82 (m, aromatic), δ 3.27 (s-2H); δ 5.01 (s-1H) ^{13}C-NMR (DMSO/400 MHz): δ 74.41, 132,158.4,195.13,201.7; m/s:475.92

ANTIBACTERIAL ACTIVITY ASSAY

Compound Tested: 8a

No of Microorganisms: 10

(*Staphylococcus aureus, Salmonella spp., E .coli, Vibrio spp., Pseudomonas aeroginosa, Vibrio parahaemolytics, Aeromonas spp., Klebsiella spp., Proteus spp. and Bacillus spp.,)*

Standard: Ampicillin (20µl/disc)

Negative Control: DMSO

PREPARATION OF INOCULUM:

Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth , that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

AGAR DISC DIFFUSION METHOD:

Antibacterial of extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc .Theplates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

Table 1-Antibacterial activity of Compound 8a

Organisms	Zone of Inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)	DMSO (20µl)
	1000	750	500		
E.coli	10mm	8mm	5 mm	20mm	-
Vibrio spp.	8 mm	5 mm	3 mm	10 mm	-
Staphylococcus aureus	15 mm	10 mm	4 mm	23mm	-
Pseudomonas aeroginosa	16mm	13mm	9mm	29 mm	-
Bacillus spp.	9 mm	6 mm	4 mm	14 mm	-
Vibrio parahaemolytics	10 mm	8 mm	5 mm	26mm	-
Salmonella spp.	**20 mm**	10 mm	8 mm	**21mm**	-
Aeromonas spp.	10 mm	7 mm	5 mm	25 mm	-
Klebsiella spp.	14mm	12 mm	8 mm	20 mm	-
Proteus spp.	12mm	10mm	9mm	20 mm	-

ANTIFUNGAL ACTIVITY ASSAY
Compound Tested: 8a

No of Microorganisms: 5
(*Candida albicans, Aspergillus flavors, Pencillium spp., Aspergillus niger and Trichophyto spp.)*

Standard: Amphotericin B (20µl/disc)

Negative Control: DMSO

PREPARATION OF INOCULUM:
Stock cultures were maintained at 4°C on Sabouraud Dextrose agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing Sabouraud Dextrose broth , that were incubated at 48hrs at 37ºC.

The Assay was performed by agar disc diffusion method.

AGAR DISC DIFFUSION METHOD:
Antibacterial of extracts was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. The disc were placed in SDA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed inthe disc .The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

Table 1-Antifungal activity of Compound 8a

Organisms	Zone of Inhibition (mm) Concentration(µg/ml)			Antibiotic (1mg/ml)	DMSO (20µl)
	1000	750	500		
Candida albicans	5 mm	3 mm	1 mm	8 mm	-
Aspergillus flavors	7 mm	5 mm	3 mm	9 mm	-
Pencillium spp.	**8 mm**	6 mm	4 mm	**7mm**	-
Aspergillus niger	**8 mm**	6 mm	3 mm	**8mm**	-
Trichophyton spp.	5 mm	4 mm	2 mm	7 mm	-

CONCLUSION

We had synthesized six novel spirooxindolinones using isatin derivatives. The synthesized compounds were characterized using UV, IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra and their structures were determined. The spiroheterocycles were subjected for testing their biological activity and all the compounds showed tremendous activity against several bacteria and fungi. Compund 8a showed appreciable activity against the bacteria *Salmonella*

spp. and against fungi *pencillium spp* and *aspergillus niger*. The significant activity of spirocompound may be attributed to the presence of aromatic rings and spirooxindole moiety.

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