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Aqueous phase microwave synthesis of some bisindolizines

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Abstract
A series of substituted bisindolizines have been synthesized in good yields using microwave irradiation of substituted phenacyl bromides, dimethyl acetylenedicarboxylate (DMAD), K_2CO_3 and 4, 4' – bipyridine in aqueous medium. All the synthesized compounds exhibited pronounced antibacterial activities. Their fluorescence studies have also been carried out.

Keywords: Antibacterial, Bisindolizine, Fluorescence, Green chemistry, Microwave synthesis.

1. Introduction
Microwave irradiation, a green chemistry technique, has been used for a variety of applications including organic synthesis. Microwave irradiation has been used as a synthetic technique for obtaining high yield, higher reaction rate and also for simplicity [1]. Using this technique, the use of toxic solvents can be eliminated and the synthesis can be carried out in a very short time period. The present study focuses on a green approach for the synthesis of bisindolizines using microwave irradiation in the aqueous reaction medium. The choice of solvent is important in green chemistry. Most of the syntheses of heterocycles have been usually carried out in polar organic solvents such as methanol, DMF and DMSO. Here the isolation and recovery of products lead to very complex procedures. Also such methods generate waste containing solvent which have to be recovered, treated and disposed off. Hence the solvent used should be nontoxic, nonhazardous and should not be harmful to the environment. All these qualities are fulfilled by water which is cheap and abundantly available [2].
Indolizines are an important class of N- fused heterocyclic compounds due to their interesting biological and optical properties. The core structure of many of the naturally occurring alkaloids is indolizine and the indolizine derivatives possess pharmacological properties like anti-inflammatory, antiviral, analgesic and antitumor activities [3-8]. A number of polycyclic indolizine and bisindolizine derivatives have been found to have long wavelength absorption and strong fluorescence in the visible region [9, 10].
The classical method for synthesis of substituted indolizines and bison designs involves a two-step reaction of quaternization of a 2- substituted pyridine/4,4'-bipyridine using an α - halo compound followed by intramolecular cyclisation with alkynes [11-14]. This procedure involves tedious steps of purification processes compared to microwave synthetic technique in aqueous medium.
In continuation to the synthesis of indolizine derivatives [12], a green aqueous phase synthetic method has been developed for eight bisindolizine derivatives. The synthesized derivatives have been studied for their fluorescence and antibacterial activities.

2. Materials and methods
Melting points were determined with a Metler melting point apparatus and are uncorrected. All reactions were carried out in a commercially available microwave oven (Samsung M183DN) operating at 300W. IR spectra were recorded on a Jasco FT/IR -4100 spectrometer using KBr. Mass spectra were recorded with a Waters-3100 spectrometer. ^{1}H NMR spectra were measured in DMSO at room temperature on Brucker Avance III 400MHz spectrometer. Elemental analyses were conducted on the Elementarvario EL III instrument. All fluorescence measurements were recorded on Jaz Ocean Optics spectrofluorometer. Thin layer chromatography was carried out on aluminium foil coated with silica gel60 F254 (Merck) and

column chromatography on silica gel; 70-230 mesh (Merck). All reagents were obtained from commercial sources and used without further purification.

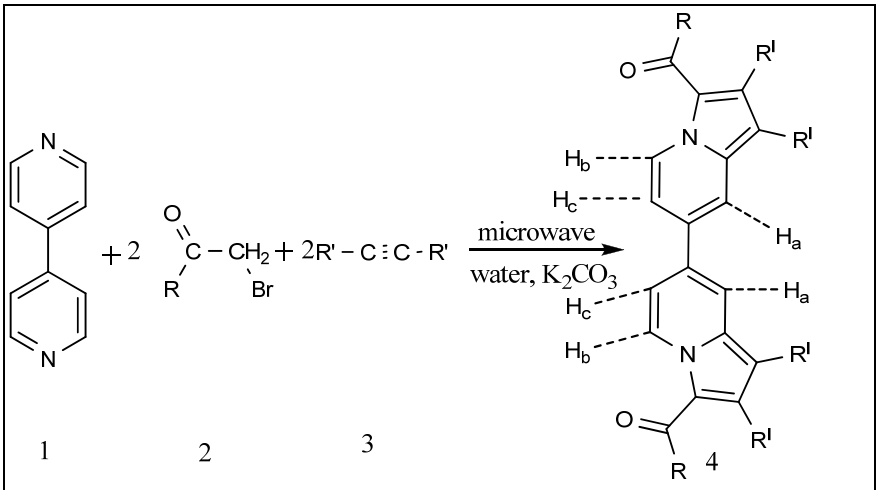
2.1 General procedure for the synthesis of bisindolizines 4a-h

4, 4'-Bipyridine (1mmol), phenacyl bromides (2 mmol), DMAD (2 mmol) and K_2CO_3 (1 g) were mixed with 1 ml of water in an Erlenmeyer flask and is fitted with a bent tube. The other end of the bent tube is connected to a receiver. The mixture was stirred and irradiated in a microwave oven at 300W for 1 minute (monitored by TLC). After cooling to room temperature, the products were extracted with methanol. The products were further purified by column chromatography using n-hexane/EtOAc, (9:1v/v). The products were recrystallized from methanol. Using the general method the Bisindolizines (4a-h) have been synthesized from 4, 4'-bipyridine (1), phenacyl bromides (2) and dimethyl acetylenedicarboxylate (3) in aqueous medium.

3. Results and Discussion

3.1 Synthesis and structure characterisation

A series of eight bisindolizines (4a-h) have been synthesized from 4, 4' -bipyridine and DMAD based on 1, 3-dipolar cycloaddition in aqueous phase. All the reactions were carried out with one equivalent of 4, 4' -bipyridine with two equivalents of phenacyl bromides and DMAD in aqueous medium. The phenacyl bromides reacted with 4, 4'-bipyridine to form ylides which *in situ* underwent cycloaddition with DMAD to form bisindolizines as described in Fig. 1. Using the above procedure 1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bisbenzoyl-7,7'-bisindolizine(4a);1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bis(p-nitrobenzoyl)-7,7'-bisindolizine(4b);1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bis(p-chlorobenzoyl)-7,7'-bisindolizine(4c);1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bis(p-methoxybenzoyl)-7,7'-bisindolizine(4d);1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bis(p-methylbenzoyl)-7,7'-bisindolizine(4e);1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bis(p-hydroxybenzoyl)-7,7'-bisindolizine(4f);1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bis(p-bromobenzoyl)-7,7'-bisindolizine(4g) and 1,2,1',2'-tetra(methoxycarbonyl)-3,3'-bis(p-fluorobenzoyl)-7,7'-bisindolizine(4h) were synthesised and the analytical data are presented in Table 1.



. Fig.1 Scheme for the synthesis of Bisindolizines(4 a-h).

Compound	R	R'
4a	Phenyl	COOMe
4b	p-nitrophenyl	COOMe
4c	p-chlorophenyl	COOMe
4d	p-methoxyphenyl	COOMe
4e	p-methylphenyl	COOMe
4f	p-hydroxyphenyl	COOMe
4g	p-bromophenyl	COOMe
4h	p-fluorophenyl	COOMe

Table 1: Analytical Data of Bisindolizines (4a-h)

Compound	Yield (%)	M.P (^{0}C)	M^+ from mass spectra	Molecular formula
4a	62	280-282	708	$C_{38}H_{28}N_2O_{10}$
4b	68	290-292	810	$C_{38}H_{26}N_4O_{14}$
4c	65	150-152	673	$C_{38}H_{26}N_2O_{10}Cl_2$
4d	57	270-272	730	$C_{40}H_{32}N_2O_{12}$
4e	75	245-247	690	$C_{40}H_{32}N_2O_{10}$
4f	51	185-187	695	$C_{38}H_{28}N_2O_{12}$
4g	68	246-248	794	$C_{38}H_{26}N_2O_{10}Br_2$
4h	62	198-200	667	$C_{38}H_{26}N_2O_{10}F_2$

The structures of compounds were confirmed by elemental analysis and spectral methods. The CHN analysis data and M+ values from mass spectra are in good agreement with the molecular formula assigned. The IR spectra of the compounds showed absorption bands between 1695 and 1710 cm^{-1} which are characteristic for ester groups. The absorption bands between 1650 and 1680 cm^{-1} indicated the presence of ketonic groups. The ^1H NMR spectra of the bisindolizines showed signals for methyl ester groups as singlets at δ 3.87- 3.95 ppm and at δ 4.05-4.13 ppm. The doublet of doublets appeared at δ 8.71-8.73 ppm is assigned to H_a proton, δ8.07- 8.13 ppm is assigned to H_b proton and the quartet appeared at δ7.72-7.79 ppm is assigned to H_c proton. Compounds (4a-h) showed a general pattern of absorption spectra in the UV-visible region consisting of three absorption bands in the range 200-210, 315-325 and 380-390 nm.

3.2 Antibacterial activity studies

Antibacterial activities of the compounds were studied by Kirby bauer method [15] against a number of microorganisms and the results obtained are presented in Table 2. The activity of the compounds was studied against *Bacillus cereus, Bacillus pumilus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Clostridium perfringens, Bacillus circulans, Pseudomonas aeruginosa, Salmonella typhimurium and Staphylococcus aureus.* Studies showed that methyl, nitro and hydroxy derivatives have pronounced activity.

Table 2: Antibacterial activity studies. + ve sign indicates activity.

Microorganism	p-methyl bisindolizine	p-nitro bisindolizine	p-hydroxy bisindolizine
B. cereus	++	+	++
B. pumilus	+	++	+
B. maculans	-	++	++
E. coli	+	++	++
Proteus vulgaris	+	++	+
Clostridium perfringens	+	++	++
Klebsiella pneumoniae	+	++	+
Pseudomonas aeruginosa	+	++	+
Salmonella typhimurium	+	++	++
Staphylococcus Aureus	+	++	+

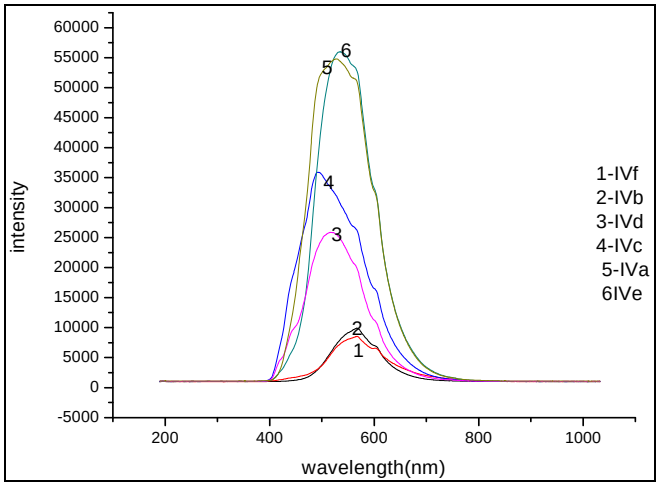


Fig 2: Fluorescence emission spectra

3.3 Fluorescence studies

Fluorescence studies of the prepared compounds were carried out and the intensity of the derivatives varies as shown in Fig. 2. Concentration of the solution used for fluorescence measurement was $5x10^{-2}$ M in methanol. Fluorescence studies showed that all the derivatives were fluorescent and methyl derivative showed maximum intensity due to the electron donating influence of methyl groups. Hydroxy derivative showed the least intense peak. Further investigation regarding solvent effects, influence of different substituents etc. is under study so as to develop applications of the synthesized derivatives.

1,2,1',2'-Tetra(methoxycarbonyl)-3,3'-bisbenzoyl-7,7'-bisindolizine 4a

Yellow crystals, Yield 62%, MP: 280-282 ^{0}C. Anal. Calcd. For $C_{38}H_{28}N_2O_{10}$: C67.86; H 4.15; N 4.16 Found C 67.79; H 4.09; N 4.06. IR (KBr cm^{-1}): 1742, 1696-1700(C=O ester) 1658(C=O ketone), 1232, 1164, 1104. ^{1}H, NMR (DMSO): δ 8.22-8.24(d, 2Hb, Jbc = 7.4Hz), 8.34-8.42 (d, 2Ha, Jac = 1.2Hz), 7.7-7.9 (dd, 2Hc, Jcb = 7.4Hz, Jca=1.2Hz), 7.3-7.61(m, 10H); 3.5(s, 6H, CH_3); 3.65(s, 6H, CH_3).

1,2,1',2'-Tetra(methoxycarbonyl)-3,3'-bis(p-nitrobenzoyl)-7,7'-bisindolizine 4b

Yellow crystals, yield 68%, MP: 290-292 ^{0}C.Anal.Calcd for $C_{38}H_{26}N_4O_{14}$: C 59.85; H 3.44; N 9.35 Found C 60.57; H 3.07; N 9.28. IR (KBr cm^{-1}) 1743, 1700-1705(C=O ester), 1668(C=O ketone), 1527, 1349, 1235, 1205. ^{1}H NMR (DMSO): δ 8.20-8.24 (d, 2Hb, Jbc = 7.2Hz); 8.50-8.54 (d, 2Ha, Jac = 1.2Hz); 7.72-7.76(dd, 2Hc,Jcb=7.2Hz,Jca=1.2Hz);820-8.24 (d, 4H, J = 7.0Hz); 7.81(d, 4H, J=7.0Hz); 3.45(s, 6H, CH_3); 3.68 (s, 6H, CH_3).

1,2,1',2'-Tetra(methoxycarbonyl)-3,3'-bis(p-chlorobenzoyl)-7,7'-bisindolizine 4c

White crystals, Yield 65%, MP: 150-152 ^{0}C. Anal.Calcd for $C_{38}H_{26}N_2O_{10}Cl_2$: C 61.55; H 3.53; N 3.78 Found C 60.57; H 3.07; N 3.28. IR (KBr cm-^{1}): 1743, 1695-1701(C=O ester), 1658(C=O ketone), 1230, 1205, 1142. ^{1}H NMR (DMSO): δ 8.22-8.24 (d, 2Hb, J bc= 7.0Hz), 8.44-8.46 (d, 2Ha, J ac= 1.2Hz); 7.68-7.72 (dd,2Hc, Jcb = 7.0Hz,Jca=1.2Hz), 8.21(d,4H,J-7.0Hz); 7.82(d,4H,J=7.0Hz); 3.45(s, 6H, CH_3); 3.65 (s,6H,CH_3).

1,2,1',2'-Tetra(methoxycarbonyl0-3,3'-bis(p-methoxybenzoyl)-7,7'-bisindolizine 4d

Yellowcrystals, Yield 57%, MP: 270-272 ^{0}C. Anal.Calcd for $C_{40}H_{32}N_2O_{12}$: C 65.57; H 4.40; N 3.82 Found C 63.57; H 4.07; N 3.28. IR (KBr cm^{-1}): 1740, 1695-1700(C=O ester), 1668(C=O ketone), 1602 1233, 1132, 1126.^{1}HNMR (DMSO):

δ 7.02-7.04 (d, 2Hb,Jbc= 7.5Hz);8.31-8.38(d,2Ha,Jac=1.3Hz);7.807.86(dd,2Hc,Jcb=7.5Hz,Jca=1.3Hz);7.31(d,4H,J=7.3Hz);6.92(d,4H,J=7.3Hz);4.14(s,6H,CH_3);3.81,(s,3H,OCH_3);3.65(s,6H,CH_3)

1,2,1',2'-Tetra(methoxycarbonyl)-3,3'-bis(p-methylbenzoyl)-7,7'-bisindolizine 4e

Reddish yellow crystals, Yield75%, MP: 245-247 ^{0}C. Anal. Calcd for $C_{40}H_{33}N_2O_{10}$: C 68.57; H 4.60; N 4 Found C 65.37; H 4.07; N 3.28. IR (KBr cm^{-1}): 1742, 1695-1702(C=O ester), 1665(C=O ketone), 1600, 1224.^{1}HNMR (DMSO): δ 7.35-7.37 (d, 2Hb,Jbc = 7.2Hz); 8.31-8.34 (d, 2Ha, Jac =1.2Hz); 7.80-7.83 (dd,2Hc,Jcb = 7.2Hz,Jca=1.2Hz)7.81-7.88(d,4H,J=7.0Hz); 7.32 7.35(d,4H,J=7.0Hz);4.12(s,6H,CH_3); 3,79(s,6H,CH_3); δ 3.45 (s,3H,CH_3).

1,2,1',2'-Tetra(methoxycarbonyl)-3,3'-bis(p-hydroxybenzoyl)-7,7'-bisindolizine 4f

Reddish yellow crystals, Yield 51%, MP: 185-187 ^{0}C. Anal. Calcd. For $C_{38}H_{28}N_2O_{12}$:C 64.77; H 4.01; N 3.98 Found C 65.21; H 4.06; N 3.24. IR (KBr cm^{-1}): 3310(b, OH), 1740, 1695-1700(C=O ester), 1680(C=O ketone), 1588, 1276, 1178. ^{1}H NMR (DMSO):7.35-7.37 (d, 2Hb,Jbc = 7.5Hz); 8.28-8.32 (d, 2Ha,Jac = 1.2Hz);7.82-7.86(dd,2Hc, Jcb=7.5Hz, Jca=1.2Hz);8.24-8.26(d,4H,J=7.0Hz);7.96-7.98(d, 4H, J-7.0Hz); 3.14(s,6H,CH_3); 3.45 (s,6H,CH_3).

1,2,1',2'-Tetra(methoxycarbonyl)-3,3'-bis(p-bromobenzoyl)-7,7'-bisindolizine 4g

Red crystals, Yield 68%, MP: 246-248 ^{0}C. Anal.Calcd for $C_{38}H_{26}N_2O_{10}Br_2$:C 54.96; H 3.16; N 3.37 Found C 52.32; H 4.59; N 3.28. IR (KBr cm-^{1}):1742, 1696-1702(C=O ester), 1658(C=O ketone), 1558, 1235, 1202.

^{1}H NMR (DMSO): δ 7.46-7.47 (d, 2Hb, Jbc = 7.2Hz); 8.34-8.38 (d, 2Ha, Jac = 1.2Hz); 7.70-7.76(dd, 2Hc,Jcb=7.2Hz,Jca=1.2Hz); 8.16-8.22 (d, 4H, J = 7.0Hz); 7.76(d, 4H, J=7.0Hz); 3.42(s, 6H, CH_3); 3.68 (s, 6H, CH_3).

1,2,1',2'-Tetra(methoxycarbonyl)-3,3'-bis(p-fluorobenzoyl)-7,7'-bisindolizine 4h

White crystals, Yield 62%, MP: 198-200 ^{0}C. Anal.Calcd for $C_{38}H_{26}N_2O_{10}F_2$: C 64.41; H 3.70; N 3.95 Found C 62.76; H 4.13 ; N 4.28 IR (KBr cm^{-1}): 1736, 1695-1701(C=O ester) 1664(C=O ketone), 1592,1390,1333,1105,1069. ^{1}H NMR (DMSO): δ 7.55-7.57 (d, 2Hb, Jbc = 7.2Hz); 8.66-8.68 (d, 2Ha, Jac = 1.2Hz); 7.79-7.86(dd, 2Hc,Jcb=7.2Hz,Jca=1.2Hz); 8.42-8.48 (d, 4H, J = 7.0Hz); 7.88(d, 4H, J=7.0Hz); 3.45(s, 6H, CH_3); 3.68 (s, 6H, CH_3).

4. Conclusion

This methodology provides access to fast one pot synthesis of bisindolizines which otherwise are accessible only through multistep synthesis. The given method of organic synthesis offers a lot of advantages connected to safety, high yield and environment friendly giving cleaner products. Microwave irradiation in aqueous phase offers a considerable improvement over classical methods. The method is very simple, easy to carry out and does not involve any tedious procedures. Here water is used as the reaction medium and the procedure is highly environment friendly. Although several other procedures are available for the synthesis of indolizines and bisindolizines, this method is highly environment friendly, easy to carry out and time saving.

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