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Mass fragmentation pattern study and Antimicrobial Study of some Benzoquinones Derivatives

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Abstract

Chemical derivatives with 1,4-benzoquinone as the basic subunit possess pharmacolo- gical activities such as antibiotic antitumor, antimalarial, anticoagulant and herbicidal properties.In spite of the extensive applications of mass spectroscopy to a wide variety of organic compounds, there are no reports on the fragmentation behaviour of substituted *p*-benzoquinone derivatives of this series. We have, for the first time, studied the mass fragmentation pattern of substituted 2, 5-dianilino-3, 6 dichloro-1, 4-benzoquinones**.**The substituted *p*-benzoquinones are potentially bioactive and they represent a class of compound with wide spectrum of biological activity of the derived benzoquinone compounds was evaluated by using Cup-Plate Agar and Kirby-Bauer Disk Diffusion method respectively. Their antibacterial activity was tested against gram-positive bacteria *Bacillus subtilis* (BS), *Micrococcus denitrificans* (MD) and gram-negative bacteria *Klebsiella aerogenes* (KA), *Pseudomonas syringae* (PS). While their antifungal activity was tested against *Aspergillus niger*, *Rizopus oryzae* and *Aspergillus flavus*. Zone of inhibition and activity index is described with respect to standard antibacterial and antifungal drugs.

Key words: 1, 4-benzoquinone, antibacterial activity, Cup-Plate Agar, Kirby-Bauer Disk Diffusion, antifungal activity, mass spectroscopy

1.INTRODUCTION

Quinones play a vital role in biological functions including oxidative phosphorylation and electron transfer [1].chemical derivatives with 1,4-benzoquinone as the basic subunit possess pharmacolo- gical activities such as antibiotic [2,3], antitumor [4-7], antimalarial [5-8], antineoplastic [9], anticoagulant [10], and herbicidal properties[11]. 3,6-Disubstituted-2,5-dimethoxy-1,4-benzo quinones are widely distributed in a large collection of natural products [12-18].They possess biological implications such as potent immunosuppressant [12], antioxidative[13], neuroprotective [14], anticoagulant [15], antidiabetic [16], anticancer [17] , and specific 5-lipoxygenase inhibitory [18] activities.

A variety of 1,4-benzoquinones and their nitrogen analogues have been reported for their antitumour activities.[19-21]. Quinones that act against animal tumours are thought to function as bio-reductive alkylating agents[22-24]. They play important role in biological functions including a role in oxidative phosphorylation and electron transfer.[25-26]

The followings of Some Substituted Benzoquinones derivatives (3a-d) shows antibacterial and antifungal activity:

(3a).3, 6-Dichloro-2, 5-dianilino-1, 4-benzoquinone (BQ I)

(3b).3,6-Dichloro-2,5-bis(2$^{'}$-methylanilino)-1,4-benzoquinone (BQ II)

(3c).3,6-Dichloro-2,5-bis(2$^{'}$-methoxyanilino)-1,4-benzoquinone (BQ III)

(3d).3,6-Dichloro-2,5-bis(2$^{'}$-fluoroanilino)-1,4-benzoquinone (BQ IV)

In the present work, antibacterial activities of the synthesized compounds were evaluated by Cup-Plate Agar Diffusion method [27].The antifungal activity of substituted benzoquinones compounds was tested by Kirby-Bauer Disk Diffusion Method [28].In spite of the extensive applications of mass spectroscopy to a wide variety of organic compounds, there are no reports on the fragmentation behaviour of substituted *p*-benzoquinone derivatives of this series. We have, for the first time, studied the mass fragmentation pattern of substituted 2, 5-dianilino-3, 6 dichloro-1, 4-benzoquinones.

2.EXPERIMENTAL METHOD

Evaluation of antibacterial activity

Various methods [29-33]are available for the evaluation of the antibacterial activity of different type of compounds. However, the most widely used method consists of determining the antibacterial activity of the drug by adding it in known concentrations to the cultures of the test organisms. In the present work, antibacterial activities of the synthesized compounds were evaluated by Cup-Plate Agar Diffusion method. [34]

Sterilization of the apparatus: All the glass apparatus were cleaned with chromic acid followed by distilled water and then sterilized by heating at 200^{0}C in a hot air oven.

Preparation of growth medium:Nutrient agar medium[35] was used as growth medium. The composition of medium used is 0.5% Peptone (5 g in 1000 ml distilled water), Beef extract (3 g), Sodium chloride (5 g), Agar agar (15 g) and Distilled water (1000 ml).

The basic steps followed for preparing the medium are listed below:

1. An appropriate amount of agar powder was suspended in 1 liter of distilled water. The agar medium was dissolved by heating to a boil. Nutrients were added to media slowly so that it dissolves completely.

2.The medium was sterilized in a validated autoclave at 1 kg/cm^{2} (15 psi) at 121^{0}C. (When medium attains a temperature of 121^{0}C for at least 15 minutes)

3.The medium was dispersed in pre-sterilized Petri plates (100 mm diameter) and kept on laminar air flow bench or stored at 0-5^{0}C until used.

Cup-Plate Agar Method:All the synthesized compounds were tested for their *in vitro* antibacterial activity against *B. subtilis*, *K. aerogenes*, *P. syringae* and *M. denitrificans* by cup-plate agar method. The sample solution was prepared by dissolving 50 µg of each of the compound in 1.0 ml of DMF. Norfloxacin (4 µg ml^{-1}) was used as standard drug for comparison. The inoculum of each of the standard bacterial strains was prepared by transferring a single well-isolated colony taken from overnight culture on nutrient agar plate in 10 ml of nutrient broth. The resultant suspension was vortexed for 15 sec. and inoculated at 37±1^{0}C for 5 hours. The turbidity was adjusted to match the 0.5 McFarland standards. Muller Hinton Agar (Hi Media) plates were inoculated with the inoculum using

a sterile cotton swab and allowed to dry for 5 min. under UV laminar flow. Cups of 6 mm diameter were made on these plates with the help of a sterile Pasteur pipette and hundred microliters of the sample solution was added in it. One cup each was inoculated with the pure solvent and standard Norfloxacin on every plate. Then, the plates were incubated aerobically at 37 ± 1^{0}C for 24 hours. After incubation, the diameter of zone of formed around each cup containing the test compound was measured accurately in millimeter using a ruler.

Evaluation of antifungal screening

Various methods[36-40]are available for the evaluation of the antifungal activity of different compounds. However, the antifungal activity of synthesized benzoquinones and triphenodioxazines compounds was tested by Kirby-Bauer Disk Diffusion Method[41].

Sterilization of the apparatus: All the glass apparatus were cleaned with chromic acid followed by distilled water and then sterilized by heating at 200^{0} C in a hot air oven.

Growth medium: Potatodextrose agar medium was used. The composition of media is as follows: Glucose (20 g), Peptone (10 g), Agar agar (15 g) and Distilled water (1000 ml).

Kirby-Bauer Disk Diffusion Method: All the synthesized compounds were studied for *in vitro* antifungal activity against the standard fungal strains of *A. niger*, *R. oryzae* and *A. flavus* using Kirby-Bauer paper disc diffusion method at two concentrations (50 and 100 μg) stock. The test solution of samples were prepared by dissolved by dissolved the DMF and was then subsequently diluted for two concentration of 50 & 100 μg ml^{-1} (with Potatodextrose agar (HI media) agar). After inculum of the agar plates with the fungal strains, the plates were dried for 10 min. under laminar flow. The dried Whattman filter paper (No. 1) discs of 6 mm diameter dipped in sample solution were placed on the medium previously seeded with the organism in Petri dishes at suitable distance. The Petri dishes containing the sample and standard griseofulvin[44] were stored in an incubator at 35 ± 1^{0}C for 48 hours. The zones of inhibition formed around each disc containing the test compound were measured accurately in millimetres.

Mass fragmentation study:

The fast atom bombardment mass spectra (FAB MS) of the substituted 2, 5-dianilino-3, 6 dichloro-1, 4-benzoquinones were recorded at room temperature on a Mass spectrometer using Aragon/Xenon as the FAB gas.

3.RESULT AND DISCUSSION

Antibacterial activity

All the p-benzoquinones derivatives were screened for their *in vitro* antibacterial activity. The minimum inhibitory concentration (MIC) method was used to assess the susceptibility of specific bacterial agents selected. The MIC test measures the lowest concentration of an antibiotic that inhibits the visible growth of test micro organisms. Abundant growth was observed in control. DMF, which has been used as solvent, showed no antibacterial activity. Inhibition was distinctly noticed after 48 hours of incubation[42].The standard strains of *Bacillus subtilis* (BS), *Klebsiella aerogenes* (KA), *Pseudomonas syringae* (PS) and *Micrococcus denitrificans* (MD) were used to determine antibacterial activity as measured by cup-plate agar diffusion method (Table 1). Compounds were dissolved in DMF at concentration of 50 μg/ml. The antibacterial activity of the test compounds were compared with standard norfloxacin (15-21mm)[43] All compounds were active against *B. subtilis*, *P. syringae* and *M. denitrificans* shown in Figure 1.Compounds 3b, were most potent and comparable to activities of standard drugs norfloxacin. While compounds 3c also possesses promising antibacterial activity. Only weak activity was observed with other compounds 3a, 3d.

Antifungal activity

All the p-benzoquinones derivatives were dissolved in DMF and tested for their in vitro antifungal activity at two concentrations of 50 and 100 µg/ml (Table 2) against *A. niger*, *R. oryzae* and *A. flavus* by the paper disc diffusion method. The minimum inhibitory concentration (MIC) method was used to assess the susceptibility of specific fungal agents selected. The MIC test measures the lowest concentration of an antibiotic that inhibits the visible growth of test micro organisms. Abundant growth was observed in control. DMF, which has been used as solvent, showed no activity. Inhibition was distinctly noticed after 48 hours of incubation[43]. The antifungal activities of the test compounds were compared to standard griseofulvin (17-23mm)[44]. All the compounds exhibited best antifungal activity against *A. niger*, least against of *R. oryzae* and moderate against *A. flavus* shown in Figure 2.Among tested, derivatives showed potent antimicrobial activity against gram-negative and gram-positive bacteria and all the strains of fungi under study. While all the compounds exhibited moderate antifungal activity against *R. orygae*. Maximum antibacterial and antifungal activity is exhibited by the compounds 3b.

Table 1 Antibacterial activity[a] of synthesized benzoquinones

Compd.	BS	KA	PS	MD
3a	+	-	++	++
3b	++++	+++	+++	++++
3c	+++	++	++	+++
3d	+	+	+	+
NF[b]	++++	++++	++++	+++
DMF	-	-	-	-

- = No measurable activity, + = 3-9mm, ++ = 10-12mm, +++ = 13-16mm, ++++ = 17-21mm, ++++ = >21mm,

[a] Data are zones of inhibition (mm), [b] Norfloxacin(Standard Drug)

Figure 1 Antibacterial activity of 3(a-d) against *Bacillus subtilis*

Table 2.Antifungal activity[a] of synthesized benzoquinone

Compd.	Inhibition of A. niger		Inhibition of R.oryzae		Inhibition of A. flavus	
	50µg/ml	100µg/ml	50µg/ml	100µg/ml	50µg/ml	100µg/ml
3a	+	++	-	+	+	+
3b	+++	++++	++	+++	++	+++
3c	++	+++	++	+++	++	+++
3d	+	+	-	-	+	+
GF[b]	++++	++++	++++	++++	++++	++++
DMF	-	-	-	-		

- = No measurable activity, + = 3-9mm, ++ = 10-12mm, +++ = 13-16mm ++++ = 17-21mm, ++++ = >21mm,
[a] Data are zones of inhibition (mm), [b]Griseofulvin(Standard Drug)

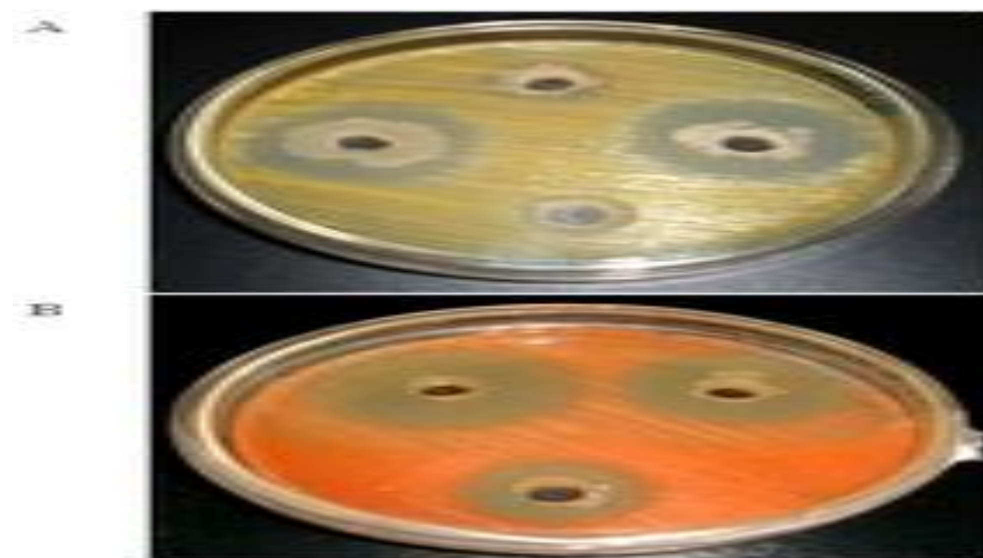


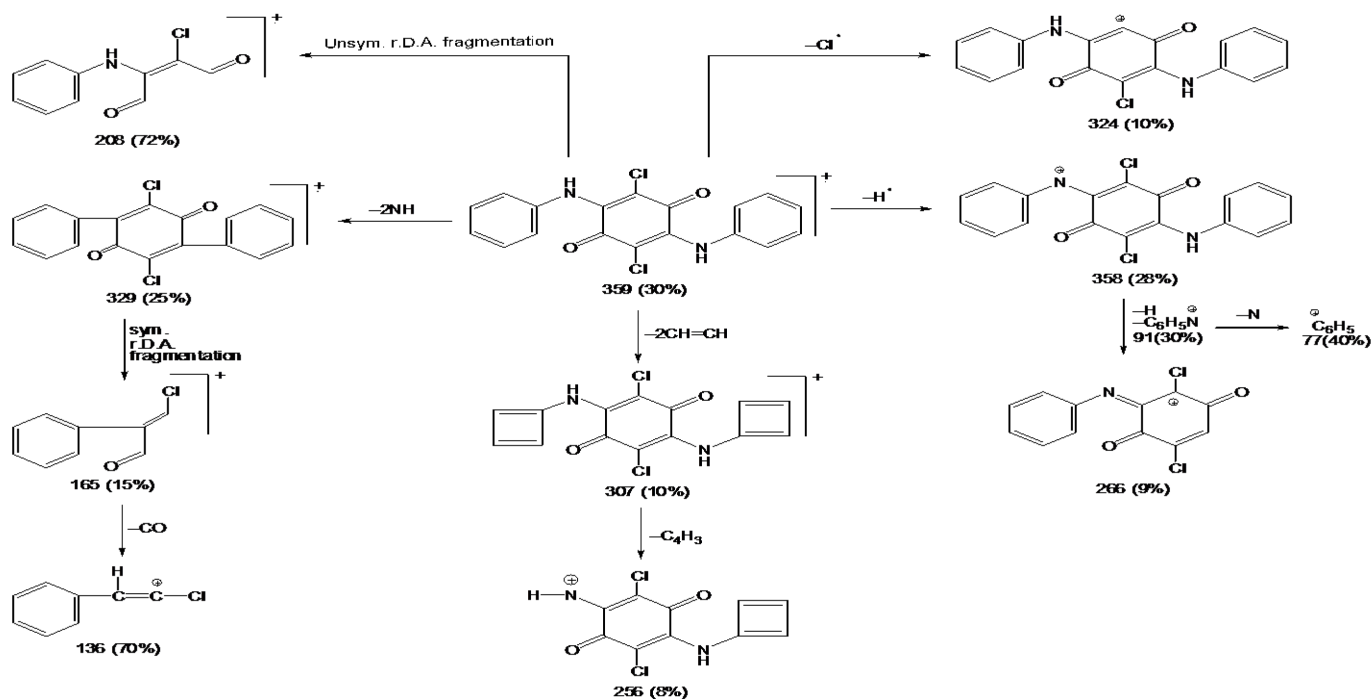
Figure 2: Antifungal activity of 3(a-d) against *Aspergillus flavus*

Fragmentation pattern of 2, 5-dianilino-3, 6-dichloro-1, 4-benzoquinone (3a)

The FAB MS of **3a** showed the molecular ion peak at m/z 359 (30%, M^+) and also isotopic abundance peak at m/z 361 (16%, M^++2) which is in accordance with the molecular weight and molecular formula of the compounds. The molecular ion (M^+) after losing one chlorine atom produces a fragment at m/z 324 (10%, M^+-Cl) along with the isotopic abundance at m/z 326 (6.5%, M^++2 -Cl). All the fragments containing two chlorine atom exhibit isotopic abundance at m/z M^+, M^++2, M^++4 in the intensity ratio of 100:65:10.6% supporting the presence of two chlorine atoms in **3a**. Fragment at m/z 358 (28%, M^+-H), formed by the loss of one proton from M^+, produces a fragment at m/z 266(9%, M^+-H-C_6H_5N) by the loss of C_6H_5N. Fragment at m/z 91 (30%, C_6H_5N) is further stabilized by the loss of N atom to generate a fragment at m/z 77 (40%, $C_6H_5^+$).The appearance of the fragment ion peak at m/z 329 (25%,

M^+ -2NH) due to elimination of two NH fragments along with fragments at m/z 358, 266, 91 and 77 supports the presence of two NH groups positioned in-between two aromatic side rings and central ring of **3a**.

Fragmentation Scheme: Fragments at m/z 329 undergoes symmetrical retro-Diels-Alder (rDA) cleavage around central ring to produce rDA fragment at m/z 165 (15%, sym. rDA frag.), which stabilizes further by losing one CO molecule to produce another fragment at m/z 136 (15%, sym. rDA frag.-CO). Similarly by the unsymmetrical rDA fragmentation of 3a around central ring a fragment at m/z 208 (72%, unsym. rDA frag.) is formed. Thus both the fragmentation pathways establish the para positioning of two CO groups in the central ring. Existence of fragment ion peak at m/z 329 along with 307(10%, M^+-$2C_2H_2$), which is formed due to loss of one acetylene fragment each from both the aromatic rings, provides sufficient evidence in favour of two anilino (C_6H_5NH) groups attached to the central ring of 3a. Thus MS fragmentation of 3a (Scheme 1) along with other spectral studies clearly suggests that two chlorine and two keto groups are attached to central ring at 3,6 and 1,4 positions, respectively, while the two anilino (C_6H_5NH) groups are attached to the central ring at the remaining 2,5 positions.



Scheme 1 Mass fragmentation pattern of 2, 5-dianilino -3, 6-dichloro-1, 4- benzoquinone

4. CONCLUSION

The minimum inhibitory concentration (MIC) method was used to assess the susceptibility of specific bacterial agent and fungal agents selected. Compounds 3b were most potent and comparable to activities of standard drugs norfloxacin. A compound 3c also possesses promising antibacterial activity. All the compounds exhibited moderate antifungal activity against *R. orygae*. Maximum antibacterial and antifungal activity is exhibited by the compounds 3b. Thus MS fragmentation of 3a (Scheme 1) along with other spectral studies clearly suggests that two chlorine and two keto groups are attached to central ring at 3,6 and 1,4 positions, respectively, while the two anilino (C_6H_5NH) groups are attached to the central ring at the remaining 2,5 positions.

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