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SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF SOME NEW 2,5-DIMETHOXY-3,6-BIS (ARYLAMINO) CYCLOHEXA-2,5-DIENE 1,4-DIONES

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ABSTRACT

A series of some new substituted 2,5-dimethoxy-3,6-bis(arylamino)cyclohexa-2,5-diene-1,4-diones has been synthesized by the condensation of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone with substituted aromatic primary amines in the presence of anhydrous sodium acetate in ethanol. The structures of newly synthesized compounds were characterized by using IR, ^{1}H NMR, Mass spectral and elemental analysis data. In addition, the antibacterial activity of these compounds has been evaluated against Gram positive and Gram negative bacteria using Streptomycin as standard drug.

Key words: 2,5-Dibromo-3,6-dimethoxy-1,4-benzoquinone, Aromatic primary amines, Condensation, Inhibition zone, Anti-bacterial activity.

INTRODUCTION

Quinones are a large class of compounds wide spread in nature[1]. They play vital role in biological functions including oxidative phosphorylation and electron transfer[2]. A large number of chemical derivatives with 1,4-benzoquinone as the basic subunit exhibit prominent pharmacological applications such as antibiotic[3,4], antitumor[5,6], antimalarial[7,8], antineoplastic[9], anticoagulant[10], and herbicidal[11] activity.

The frame work of 3,6-disubstituted-2,5-dimethoxy-1,4-benzoquinone and its reductive phenolic forms are widely distributed in a large collection of natural products. They displayed a broad spectrum of biological activities such as potent immunosuppressant[12], antioxidative[13], neuroprotective[14], anticoagulent[15], antidiabetic[16], anticancer[17], and specific 5-lipoxygenase inhibitory[18], activities.

A few aryl amino-1,4-benzoquinones such as substituted-5-(arylamino)-2-hydroxy-3-undecyl-1,4 -benzoquinones possess antioxidant, anti-inflammatory, analgesic activities[19]. 2,5-Diaminoaryl- 3,6-dibromo-1,4-benzoquinones and 2,5-diamino(N-alkyl and N-heteroaryl)-3,6-dibromo-1,4-benzoquinones exhibit antibacterial and antifungal activities[20]. 3,6-Dihalo-2,5-bis(2,4,5,-trichloroanilino)-1,4-benzoquinones are known to have antibacterial activity[21].

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In view of the broad range of biological activities of substituted arylamino-1,4-benzoquinone derivatives, a new series of 2,5-dimethoxy-3,6-bis(substituted arylamino)-2,5-diene-1,4-diones was synthesized and their antibacterial activity was tested against Gram positive and Gram negative bacteria.

EXPERIMENTAL

Materials and methods

All the reagents and solvents used were of laboratory grade. Melting points of all the compounds are determined in open capillary method and are uncorrected. All the new products are monitored by TLC using Merck brand silica gel-G plates for TLC. IR spectra are recorded on Nexus 470 FTIR Spectrometer. ^{1}H NMR spectra are recorded on Varian Mercury 400MHz spectrometer using TMS as internal standard. Mass spectra are obtained on Shimadzu mass spectrometer. In the present work various substituted 2,5-dimethoxy-3,6-bis(arylamino)-1,4-benzoquinones are synthesized from 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone by the reaction with substituted aromatic primary amines in ethanol in presence of fused sodium acetate.

Preparation of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone (4)

2,5-Dihydroxy-1,4-benzoquinone (**2**) was prepared by the oxidation of hydroquinone (**1**) according to the literature[22]. The two hydroxyl groups of compound **(2)** are then protected by reaction with methanol under acidic condition to give 2,5-dimethoxy-1,4-benzoquinone (**3**) and bromination of quinone **(3)** with N-bromosuccinimide (NBS) afforded 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone (**4**) by reported method[23]. The **(4)** was identified by spectroscopic data.

General procedure

Synthesis of 2,5-dimethoxy-3,6-bis(substituted arylamino)-cyclohexa-2,5-diene-1,4-diones (6a-j)

3.068 mmol of anilines (**5a-j**) was taken into a 50 mL round bottom flask and 15 mL of ethanol was added followed by anhydrous sodium acetate (3.068 mmol). It was stirred for 15 minutes at room temperature and subsequently 1.534 mmol of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone **(4)** was added portion wise. The reaction mixture was heated at reflux temperature for 3-5 hrs. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature. The precipitate formed was filtered, first washed with hot distilled water and then 30% ethanol. After washing several times with petroleum ether, the products were recrystallized from ethanol. The synthetic approach is shown in **Scheme 1**.

The following compounds are synthesized by using general procedure.

2,5-Dimethoxy-3,6-bis(phenyl amino)cyclohexa-2,5-diene-1,4-dione (6a)

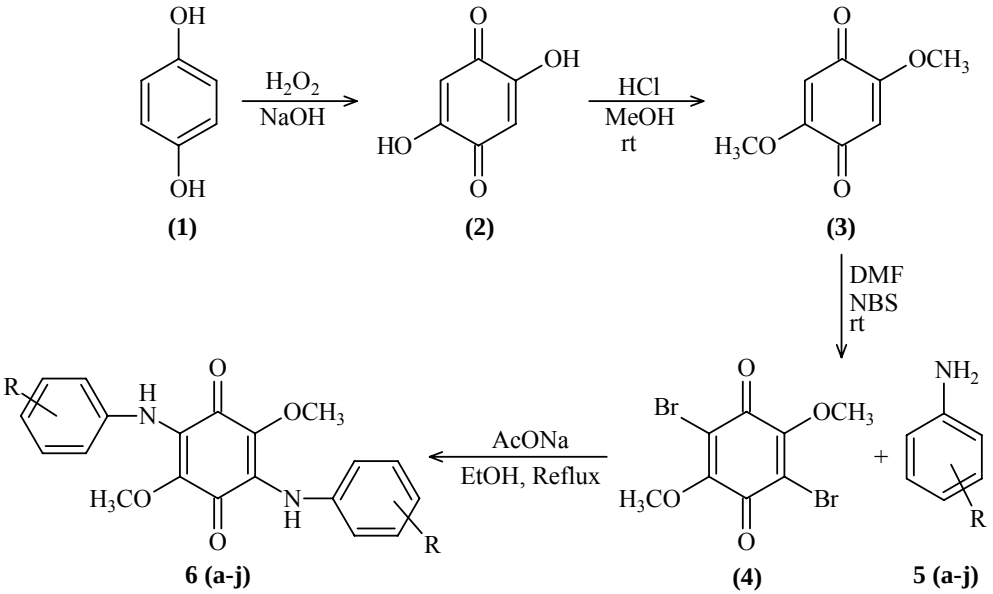
Yield: 435 mg (81%), m.p. 251-253^{o}C; **IR (KBr)** ν_{max} cm^{-1}: 3233 (NH), 1647 (C=O), 1600 (N-H, bend), 1310 (C-N), 1245, 1055 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d$_6$) δ: 8.08 (2H, br.s, NH), 6.72-7.12 (10 H, m, Ar-H), 3.71 (6H, s,OCH$_3$). **MS**, m/z 351 [M+H]$^+$. Anal. Calcd for $C_{20}H_{18}N_2O_4$ C, 68.47; H, 5.09; N, 8.18. Found C, 68.39; H, 5.12; N, 8.10.

2,5-Bis(4-bromophenylamino)-3,6-dimethoxy cyclohexa-2,5-diene-1,4-dione (6b)

Yield: 538mg (69%), m.p. 282-284^{0}C; **IR (KBr)** ν_{max}cm^{-1}: 3236 (NH), 1642 (C=O), 1586 (NH, bend) 1329 (C-N), 1238, 1042 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d$_6$) δ: 8.04 (2H, br.s, NH), 6.69-7.24 (8H, m, Ar-H), 3.73 (6H, s, OCH$_3$). **MS**, m/z 507 [M+H]$^+$. Anal. Cacld for $C_{20}H_{16}Br_2N_2O_4$C, 47.31; H, 3.20; N, 5.44. Found C, 47.34; H, 3.17; N, 5.49.

2,5-Bis(4-fluorophenylamino)-3,6-dimethoxy cyclohexa-2,5-diene-1,4-dione (6c)

Yield: 373 mg (63%), m.p. 228-230^{o}C; **IR (KBr)** ν_{max} cm^{-1}: 3230 (NH), 1644 (C=O), 1585 (NH, bend), 1332 (C-N), 1236, 1058 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 7.98 (2H, br.s, NH), 6.65-6.95 (8H, m, Ar-H), 3.75 (6H, s, OCH_3). **MS**, m/z 387 $[M+H]^+$. Anal. Calcd for $C_{20}H_{16}F_2N_2O_4$C, 62.21; H, 4.09; N, 7.30. Found C, 62.28; H, 4.03; N, 7.33.



(a) R = H; (b) R = 4-Br; (c) R = 4-F; (d) R = 4-Cl; (e) R = 4-CH_3;
(f) R = 4-OCH_3; (g) R = 4-NO_2; (h) R = 4-NO_2; (i) R = 2-NO_2; (j) R = 4-NH_2

Scheme 1

2,5-Bis(4-chlorophenylamino)-3,6-dimethoxy cyclohexa-2,5-diene-1,4-dione (6d)

Yield: 431mg (67%), m.p. 277-279^{o}C; **IR (KBr)** ν_{max} cm^{-1}: 3238 (NH), 1642 (C=O), 1585 (NH, bend), 1331 (C-N), 1234, 1042 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.01 (2H, br.s, NH), 6.77-7.01 (8H, m, Ar-H), 3.75 (6H, s, OCH_3). **MS** m/z 419 $[M+H]^+$. Anal.calcd for $C_{20}H_{16}Cl_2N_2O_4$, C, 57.20; H, 3.80; N, 6.51. Found C, 57.15; H, 3.76; N, 6.48.

2,5-Dimethoxy-3,6-bis(p-tolylamino)cyclohexa-2,5-diene-1,4-dione (6e)

Yield: 500 mg(86%), m.p. 217-219^{0}C; **IR (KBr)** ν_{max} cm^{-1}: 3229 (NH), 1642 (C=O), 1585 (NH, bend), 1315 (C-N), 1250, 1047 (C-O-C). **^{1}H NMR** (400 MHz DMSO-d_6) δ: 7.98 (2H, br.s, NH), 7.02-6.59 (8H, m, Ar-H), 3.68 (6H, s, OCH_3), 2.21 (6H, s, CH_3). **MS**, m/z 379 $[M+H]^+$ Anal. Calcd for $C_{22}H_{22}N_2O_4$, C, 69.92; H, 5.90; N, 7.51. Found C, 69.96; H, 5.84; N, 7.54.

2,5-Dimethoxy-3 6-bis(4-methoxyphenylamino)cyclohexa-2,5-diene-1,4-dione (6f)

Yield: 554 mg (88%), m.p. 263-264^{o}C; **IR (KBr)** ν_{max} cm^{-1}: 3225 (NH), 1641 (C=O), 1580 (NH, bend), 1326 (C-N), 1232, 1045 (C-O-C). **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 7.96 (2H, br.s, NH), 6.64-6.71 (8H, m, Ar-H), 3.80 (6H, s, OCH_3), 3.71 (6H, s, OCH_3). **MS**, m/z, 411 $[M+H]^+$ Anal. Calcd for $C_{22}H_{22}N_2O_6$, C, 64.35; H, 5.50; N, 6.74. Found C, 64.31; H, 5.44; N, 6.85.

2,5-Dimethoxy-3,6-bis(3-nitrophenyl amino)cyclohexa-2,5-diene-1,4-dione (6g)

Yield: 465 mg (69%), m.p. 235-237°C; **IR (KBr)** ν_{max} cm^{-1}: 3240 (NH), 1650 (C=O), 1620 (NH, bend), 1515, 1335 (NO_2), 1321 (C-N), 1265, 1051, (C-O-C). **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.09 (2H, br.s, NH), 7.14-7.61 (8H, m, Ar-H), 3.79 (6H, s, OCH_3). **MS**, m/z, 441 $[M+H]^+$. Anal. Calcd for $C_{20}H_{16}N_4O_8$, C, 54.05; H, 3.81; N, 12.99. Found C, 53.97; H, 3.78; N, 13.03.

2,5-Dimethoxy-3,6-bis(4-nitrophenylamino)cyclohexa-2,5-diene-1,4-dione (6h)

Yield: 432 mg (64%), m.p. 198-200°C; **IR (KBr)** ν_{max} cm^{-1}: 3243 (NH), 1652 (C=O), 1622 (NH, bend), 1520, 1338 (NO_2), 1324 (C-N), 1265, 1056 (C-O-C); **^{1}H NMR** (400 MHz, DMSO-d_6) δ: 8.18 (2H, br.s, NH), 6.97-7.92 (8H, m, ArH), 3.74 (6H, s, OCH_3). **MS**, m/z, 441 $[M+H]^+$. Anal. Calcd for $C_{20}H_{16}N_4O_8$, C, 54.05; H, 3.81; N,12.99. Found C, 53.97; H, 3.78; N, 13.03.

2,5-Dimethoxy-3,6- bis (2-nitrophenylamino) cyclohexa-2, 5-diene-1, 4-dione (6i)

Yield: 410mg (61%), m.p. 212-214°C; **IR (KBr)** ν_{max} cm^{-1}: 3243 (NH), 1652 (C=O), 1625 (NH, bend), 1512, 1335 (NO_2), 1341 (C-N), 1263, 1058 (C-O-C). **^{1}H NMR** (400 MHz, DMSO-d_6), δ: 8.25 (2H, s, NH), 7.12-8.07 (8H, m, Ar-H), 3.78 (6H, s, OCH_3). **MS**, m/z, 441$[M+H]^+$ Anal. Calcd for $C_{20}H_{16}N_4O_8$, C, 54.05; H, 3.81; N, 12.99. Found C, 53.97; H, 3.78; N, 13.03.

Table 1: Physical data of compounds (6a-j)

Compd.	R	M.P. (°C)	Yield (%)	Molecular formula
6a	H	251-253	81	$C_{20}H_{18}N_2O_4$
6b	4-Br	282-284	69	$C_{20}H_{16}Br_2N_2O_4$
6c	4-F	228-230	63	$C_{20}H_{16}F_2N_2O_4$
6d	4-Cl	277-279	67	$C_{20}H_{16}Cl_2N_2O_4$
6e	4-CH_3	217-219	86	$C_{22}H_{22}N_2O_4$
6f	4-OCH_3	263-264	88	$C_{22}H_{22}N_2O_6$
6g	3-NO_2	235-237	69	$C_{20}H_{16}N_4O_8$
6h	4-NO_2	198-200	64	$C_{20}H_{16}N_4O_8$
6i	2-NO_2	212-214	61	$C_{20}H_{16}N_4O_8$
6j	4-NH_2	211-213	73	$C_{20}H_{20}N_4O_4$

2, 5-Bis (4-aminophenylamino)-3, 6-dimethoxy-cyclohexa-2, 5-diene-1, 4-dione (6j)

Yield: 425 mg (73%), mp 211-213°C; **IR (KBr)** ν_{max} cm^{-1}: 3463, 3374 (NH_2), 3246 (NH), 1650 (C=O), 1608 (NH, bend), 1335 (C-N), 1265, 1051 (C-O-C). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.95 (2H, br.s, NH), 6.13-6.49 (8H, m, ArH), 3.84 (4H, br.s, NH_2), 3.72 (6H, s, OCH_3). **MS**, m/z, 381 $[M+H]^+$, Anal. Calcd for $C_{20}H_{20}N_4O_4$ C,63.21; H, 5.22; N, 14.68. Found C, 63.14; H, 5.28; N, 14.66.

Antibacterial activity

All the synthesized compounds (**6a-j**) were screened for antibacterial activity according to agar disc diffusion[24] method against Gram-positive bacteria *Staphylococcus aureus, Micrococcus luteus, Bacillus cereus* and Gram-negative bacteria *Escherichia coli, Enterobacter aerogens, Salmonella paratyphi,*

Klebsiella pheumonea using Streptomycin as standard drug. The compounds were tested at 400, 600, 800 µg/mL concentrations and diameter of the inhibition zone (DIZ) was measured in mm.

The results of the antibacterial activity of the compounds (**6a-j**) are shown in the Table 2. By the analysis of the data, it was found that majority of the synthesized compounds showed varying degrees of inhibition against the tested bacteria. The compound (**6c**) showed good activity against Gram-positive bacteria and the compound (**6d**) showed excellent activity against Gram-negative bacteria. Compounds (**6g-i**) showed good activity against both the bacterial strains. Among (**6g -i**), compound (**6h**) showed high degree of inhibition.

Table 2: Antibacterial activity data

Antibacterial activity of the compounds (6a-j)																					
Compd.	**Zone of inhibition in mm**																				
	E. coli			***E. aerogenes***			***S. paratyphi***			***K. pneumoniae***			***S. aureus***			***B. cereus***			***M. luteus***		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
6a			10					10	10		10	10	10	10	12	9	10	10	10	12	12
6b		10	12		10	12	10	12	12		10	10	14	14	16	12	14	15	12	12	14
6c	10	12	14			12	12	12	13	10	11	13	16	18	19	18	18	20	17	17	19
6d	20	21	22	20	22	22	19	22	23	21	23	23	12	14	14	13	13	14	12	12	14
6e	15	16	18		10	12	10	12	13	16	18	19	14	15	16	16	17	19	10	12	14
6f	10	12	13	12	13	15	16	17	19	10	12	13	10	12	14	12	13	14	10	12	15
6g	16	18	19	16	17	18	17	17	19	16	18	18	14	15	16	15	15	16	15	16	18
6h	16	17	18	16	18	19	18	18	20	16	18	19	20	22	23	19	20	22	20	21	22
6i	14	15	17	14	16	18	15	16	17	16	16	18	15	16	19	14	16	18	16	17	19
6j	13	14	16	15	15	16	14	15	17	14	15	16	10	12	14			12		12	16
Strepto-mycin (Standard)	30	32	32	30	30	32	29	30	32	30	30	32	28	29	30	30	32	32	28	30	32
Test solution and Standard solution **A** : 400 µg/mL; B : 600 µg/mL; **C** : 800 µg/mL																					

RESULTS AND DISCUSSION

In the present work, compounds (**6a-j**) are synthesized by the condensation of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone (**4**) with substituted aromatic primary amines (**5a-j**) in ethanol in presence of fused AcONa. The purity and homogeneity of all the synthesized compounds were confirmed by their sharp melting points (uncorrected) and thin layer chromatography. The structures of all the synthesized compounds (**6a-j**) have been supported by elemental analysis and their spectral data.

Elemental analysis revealed the presence of two nitrogens introduced through formation of 2,5-dimethoxy-3,6-bis(substituted arylamino)-1,4-benzoquinones (**6a-j**). New absorptions showed up in the

range 3225-3246, 1580-1625 and 1310-1341 cm^{-1}, respectively for the aromatic secondary amine N-H stretching, N-H bending and C-N stretching in the infrared spectrum, confirmed the linkage of two aromatic primary amines links to the quinone system. Further, evidence has also been provided by the proton magnetic resonance spectrum, which has a broad singlet peak in the range δ 7.95-8.25 accounting for two hydrogens pertaining to protons attached to -NH- in the part of aromatic secondary amine unit. The peaks in the aromatic region between δ 6.13-8.07 show the presence of phenyl ring protons. The M+1 peak of compounds in the mass spectrum with reasonable intensity gave evidence for the molecular weight of the compounds. The fragmentation pattern observed was is in accordance with the literature proposed.

CONCLUSION

Different 2,5-dimethoxy-3,6-bis(substituted arylamino) cyclohexa-2,5-diene-1,4-diones **(6a-j)** were successfully prepared by the condensation of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone with substituted aromatic primary amines in the presence of anhydrous sodium acetate in ethanol. All the products were found in good yield. The synthesized compounds showed varying degrees of inhibition against both the bacterial strains

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