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Synthesis, characterization and antibacterial activity of novel 2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)diacetic acid analogues

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ABSTRACT

A series of some new 2,2'(2,5-dimethoxy-3,6-dioxocycloxa-1,4-diene-1-4-diyl)bis(azanediyl) diacetic acid analogues were synthesized with the objective for evaluation as antimicrobials. Reaction of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone with amino acids in the presence of triethylamine in ethanol gave the corresponding title compounds. The structures of all the newly synthesized compounds have been supported by elemental analysis, IR, ^{1}H NMR and mass spectral data. All the synthesized compounds were tested for their antibacterial activity in comparison with the standard drug Streptomycin.

Key words: 2,5-Dibromo-3,6-dimethoxy-1,4-benzoquinone, Amino acid, Condensation, Inhibition zone, Antibacterial activity

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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. Introduction of different amino acid moieties into the quinonoid system leads to possession of prominent biological activities [19-25]. Naturally occurring 1,4-benzoquinone-amino acid conjugates were isolated from the roots of *Embelia ribes* [26] and marine sponges species[27, 28]. They possess analgesic, anti-inflammatory, antioxidant, antitumor**,** antifertility, cytotoxic, antimicrobial and antiviral activities. Several researchers reported [29-31] the synthesis and potential activities of 1,4-benzoquinone-amino acid conjugates.

In view of the broad range of biological activities of quinone-amino acid conjugates, we synthesized various novel 2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis- (azanediyl)diacetic acid analogues from 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone by condensation with various amino acids using triethylamine as base in ethanol solvent.

EXPERIMENTAL SECTION

All the reagents and solvents used were of laboratory grade. Melting points of all the compounds are determined in open capillary tubes using Sis co melting point apparatus and are uncorrected. The progress of the reaction and purity of all the new products were monitored by TLC using Merck brand silica gel-G plates. IR spectra are recorded on Nexus 470 FTIR spectrometer, ^{1}H NMR spectra are recorded on Varian mercury 400 MHz spectrometer

in DMSO using tetramethyl silane (TMS) as an internal standard. Mass spectra are obtained on Shimadzu mass spectrometer.

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 reported literature [32]. The two hydroxyl groups of the compound **2** are then protected by the reaction with methanol under acidic conditions to give 2,5-dimethoxy-1,4-benzoquinone **3.** Bromination of 2,5-dimethoxy-1,4-benzoquinone **3** with N-bromosuccinimide (NBS) afforded 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone **4** by reported method [33] and identified by spectroscopic data.

General procedure for synthesis of 2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl) diacetic acid analogues (6a-j)
3.068 mmol of amino acid **5** was taken into a round bottom flask, added 15 ml of ethanol and cooled to 0-5^{0}C temperature. Then 6.136 mmol of triethylamine (Et_3N) was added slowly drop wise, temperature maintained at 0-5^{0}C for 30 minutes. 1.534 mmol of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone **4** was then added portion wise to the above reaction mixture. The reaction mixture was slowly raised to reflux temperature and maintained it for 3-4 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to 10-15^{0}C, poured into ice cold distilled water, then adjusted to P^H 5-6 with 1N HCl and extracted with ethylacetate (3x20ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was subjected to column chromatography using variants of ethyl acetate-petroleum ether mixture. Concentrated the fractions containing the compound by distilling out the solvent to obtain the pure brown colour crystalline solid (**Scheme1**).

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)diacetic acid (6a)
Yield: 0.41 g, 85%, brown colour solid, mp 109-111^{0}C. I.R (KBr) ν_{max} cm^{-1}: 3447(NH), 3088(OH), 1710(C=O, COOH), 1658(C=O), 1228, 1037(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 11.22(2H, br.s, OH), 7.70(2H, br.s, NH), 4.18(4H, s, CH2), 3.75(6H, s, OCH3). MS, m/z 315 [M+H]$^+$. Anal. Calcd. for $C_{12}H_{24}N_2O_8$, C, 45.74; H, 4.51; N, 9.01. Found C, 45.79; H, 4.44; N, 8.96.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)bis(2-phenyl acetic acid) (6b)
Yield: 0.515 g, 72%, brown colour solid, mp 205-207^{0}C. IR (KBr) ν_{max} cm^{-1}: 3432(NH), 3072(OH), 1700(C=O, COOH), 1652(C=O), 1237, 1045(C-O-C). ^{1}H NMR (400 MHz DMSO): δ 11.07(2H, br.s, OH), 7.31-7.42(10H, m, ArH), 7.16(2H, br.s, NH), 5.29(2H, s, CH), 3.76(6H, s, OCH_3). MS, m/z 467 [M+H]$^+$. Anal. Calcd. for $C_{24}H_{22}N_2O_8$, C, 61.76; H, 4.81; N, 5.94. Found C, 61.87; H, 4.73; N, 6.04.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)dipropanoicacid (6c)
Yield: 0.410g, 78%, brown colour solid, mp 104-106^{0}C. IR (KBr) ν_{max} cm^{-1}: 3432(NH), 3081(OH), 1714(C=O, COOH), 1648(C=O), 1225, 1021(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 11.05(2H, s, OH), 7.56(2H, br.s, NH), 3.94(2H, m, CH), 3.78(6H, s, OCH_3), 1.58(6H, d, CH_3). MS, m/z 343[M+H]$^+$. Anal.Calcd. for $C_{14}H_{18}N_2O_8$, C, 49.23; H, 4.28; N, 8.25. Found C, 49.10; H, 5.35; N, 8.14.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)bis(3-phenyl- propanoic acid) (6d)
Yield: 0.568g, 75%, brown colour solid, mp 228-230^{0}C. IR (KBr) ν_{max} cm^{-1}: 3428(NH), 3065(OH), 1703(C=O, COOH), 1650(C=O), 1236, 1048(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 10.98(2H, br.s, OH), 7.26-7.24(10H, m, ArH), 6.68(2H, br.s, NH), 4.16(2H, t, CH), 3.73(6H, s, OCH_3), 3.16(4H, d, CH_2). MS, m/z 495 [M+H]$^+$. Anal. Calcd. for $C_{26}H_{22}N_2O_8$, C, 63.02; H, 5.21; N, 5.71. Found C, 62.07; H, 5.26; N, 5.68.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)dibutanoic acid (6e)
Yield: 0.46g, 81%, brown colour solid, mp 124-126^{0}C. IR (KBr) ν_{max} cm^{-1}: 3420(NH), 3069(OH), 1708(C=O, COOH), 1645(C=O), 1231, 1041(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 10.95(2H, br.s, OH), 7.68(2H, br.s, NH), 3.77(6H, s, OCH_3), 3.72(2H, t, CH), 1.69(4H, m, CH_2), 0.90(6H, t, CH_3). MS, m/z 371 [M+H]$^+$. Anal. Calcd for $C_{16}H_{22}N_2O_8$, C, 51.96; H, 6.04; N, 7.43. Found C, 51.82; H, 5.98; N, 7.52.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)bis(3-methylbutanoic acid) (6f)
Yield: 0.427g, 70%, brown colour solid, mp 115-117^{0}C. IR (KBr) ν_{max} cm^{-1}: 3420(NH), 3056(OH), 1705(C=O, COOH), 1642(C=O), 1223, 1042(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 11.08(2H, br.s, OH), 7.78(2H, br.s, NH), 3.81(2H, d, CH), 3.76(6H, s, OCH_3), 1.73(2H, m, CH), 0.99(12H, d, CH_3). MS, m/z 399 [M+H]$^+$. Anal. Calcd. for $C_{18}H_{28}N_2O_8$, C, 54.30; H, 6.67; N, 6.97. Found C, 54.34; H, 6.61; N, 7.01.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)bis(4-methyl- pentanoic acid) (6g)
Yield: 0.47g, 72%, brown colour solid, mp 139-141^{0}C. IR (KBr) ν_{max} cm^{-1}: 3418(NH), 3058(OH), 1706(C=O, COOH), 1644(C=O), 1221, 1040(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 10.92(2H, br.s, OH), 7.95(2H, br.s, NH), 3.76(6H, s, OCH_3), 3.70(2H, t, CH), 1.74(6H, m, CH_2-CH), 0.91(12H, d, CH_3). MS, m/z 427 $[M+H]^+$. Anal. Calcd for $C_{20}H_{30}N_2O_8$, C, 56.26; H, 7.15; N, 6.66. Found C, 56.34; H, 7.17; N, 6.54.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)bis(3-methyl pentatonic acid) (6h)
Yield: 0.484g, 74%, brown colour solid, mp 118-120^{0}C. IR (KBr) ν_{max} cm^{-1}: 3414(NH), 3062(OH), 1704(C=O, COOH), 1643(C=O), 1220, 1038(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 10.95(2H, br.s, OH), 8.04(2H, br.s, NH), 3.77(6H, s, OCH_3), 3.74(2H, d, CH), 1.80(2H, m, CH), 1.36(4H, m, CH_2), 0.99(6H, d, CH_3), 0.87(6H, d, CH_3). MS, m/z 427 $[M+H]^+$. Anal. Calcd. for $C_{20}H_{30}N_2O_8$, C, 56.26; H, 7.15; N, 6.66. Found C, 56.34; H, 7.17; N, 6.54.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)bis(4-(methylthio) butanoic acid) (6i)
Yield: 0.44g, 62%, brown colour solid, mp 149-151^{0}C. IR (KBr) ν_{max} cm^{-1}: 3419(NH), 3064(OH), 1702(C=O, COOH), 1650(C=O), 1232, 1044(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 11.30(2H, br.s, OH), 8.08(2H, br.s, NH), 3.79(6H, s, OCH_3), 3.74(2H, d, CH), 2.63(4H, t, CH_2), 2.12(6H, s, CH_3), 1.98(4H, m, CH_2). MS, m/z 463 $[M+H]^+$. Anal. Calcd for $C_{18}H_{26}N_2O_8S_2$, C, 46.88; H, 5.73; N, 5.99. Found C, 46.77; H, 5.69; N, 6.09.

2,2'-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(azanediyl)bis(3-(4 hydroxy phenyl) propanoic acid(6j)
Yield: 0.557g, 69%, brown colour solid, mp 231-233^{0}C. IR (KBr) ν_{max} cm^{-1}: 3425(NH), 3244(OH), 3060(OH), 1705(C=O, COOH), 1652(C=O), 1234, 1047(C-O-C). ^{1}H NMR (400 MHz, DMSO): δ 10.92(2H, br.s, OH), 7.59(2H, br.s, NH), 6.54(4H, d, ArH), 7.13(4H, d, ArH), 5.01(2H, br.s, OH), 4.12(2H, t, CH), 3.66(6H, s, OCH_3) 2.95(4H, d, CH_2). MS, m/z 527 $[M+H]^+$. Anal. Calcd for $C_{26}H_{26}N_2O_{10}$, C, 59.42; H, 4.96; N, 5.41. Found C, 59.36; H, 5.01; N, 5.35.

Antibacterial Activity
The Antibacterial activity of the synthesized compounds (**6a-j**) were evaluated according to agar disc diffusion method [34] against gram negative bacteria *Escherichia coli*, *Salmonella paratyphi*, *Klebsiella pneumonia* and Gram positive bacteria *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus* with standard drug Streptomycin.

Table 1 Antibacterial Activity of the compounds (6a-j)

S.No	Compound	Zone of inhibition (in mm)																	
		E.Coli			*Salmonella paratyphi*			*Klebsiella pneumoniae*			*Staphylococcus aureus*			*Micrococcus luteus*			*Bacillus cereus*		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	6a	18	20	21	24	25	25	16	22	22	16	18	22	20	24	24	18	22	22
2	6b	18	19	20	20	20	20	17	21	21	18	20	20	19	20	22	18	21	21
3	6c	19	20	21	16	19	19	16	20	20	13	17	18	15	18	19	16	19	19
4	6d	16	20	20	16	17	17	17	17	17	16	17	17	18	19	19	14	17	18
5	6e	23	26	26	27	28	28	21	23	23	20	24	27	21	22	24	21	24	28
6	6f	14	18	18	10	15	16	12	14	15	12	13	15	16	20	21	10	12	12
7	6g	15	16	16	14	22	23	13	16	17	19	21	21	18	20	23	24	19	20
8	6h	22	23	23	25	26	26	17	17	18	20	23	26	20	23	23	18	21	25
9	6i	24	24	24	21	23	23	20	23	23	20	23	23	23	24	24	22	22	22
10	6j	23	26	26	25	26	26	22	23	23	24	26	26	24	25	25	23	22	25
11	Streptomycin	28	30	30	28	28	30	30	30	31	28	29	30	28	30	32	29	30	30

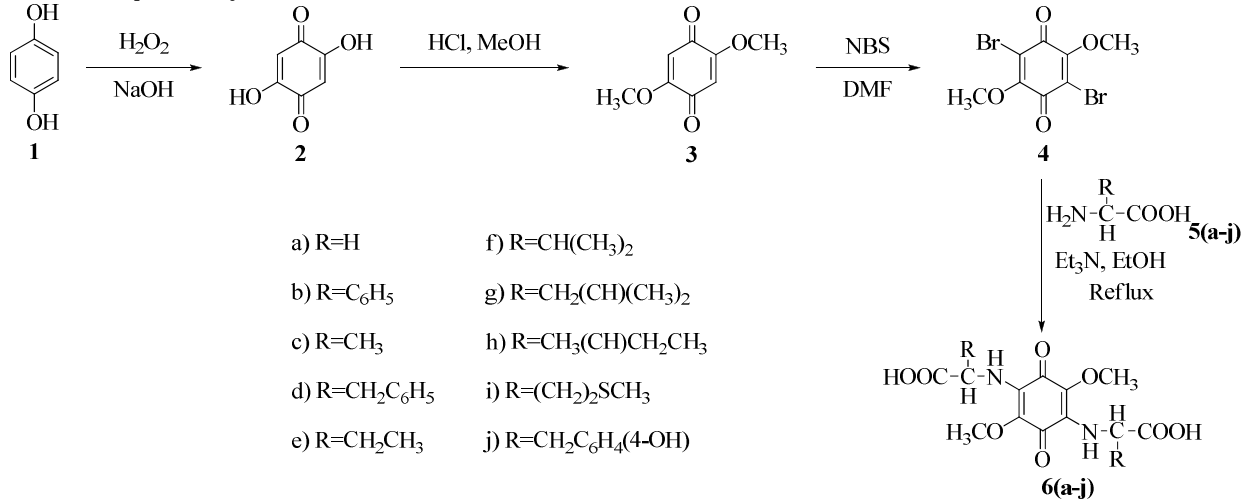
Test solution and Standard Solution; A: 400 µg/ml; B: 600 µg/ml; C: 800 µg/ml

20 ml of the molted agar medium was poured in each of the sterilized petridishes and cooled to 45-48^{0}C. For bioassays, a suspension of approximately 1.5x10^8 bacterial cells/ml was prepared as described by Forbes et al., [35] and 1.5 ml of it was uniformly spreaded on nutrient agar media. The plates were left to stand for 1 hour to solidify. After solidification of the medium, cups (wells) were made about 2 cm apart using sterile cork borer at equal distances. 0.2 ml of respective concentration of the test compound solution in dimethyl sulfoxide (DMSO) was added to each hole. The plates were allowed to stand at room temperature for one hour to allow the solution to diffuse into the medium and then incubated at 37^{0}C for 18 hours. After incubation period bioactivity was determined by measuring diameter of the inhibition zone (DIZ) in mm. Controls included the use of solvent without test sample. The experiment was performed three times with 400, 600 and 800 µg/ml concentrations.

RESULTS AND DISCUSSION

The synthesis of compounds (**6a-j**) was carried out by the reaction of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone with amino acids in the presence of triethylamine in ethanol (scheme 1). The purity and homogeneity of all the synthesized compounds were confirmed by their sharp melting points and thin layer chromatography.

Elemental analysis revealed the presence of two nitrogens introduced through formation of 1,4-benzoquinone-amino acid conjugate. New absorption showed up at 3447, 3088 and 1710 respectively for the secondary amine and hydroxyl and carbonyl groups of carboxylic acid in the infrared spectrum confirmed the linkage of two amino acid units to the quinone system.



Scheme 1

Further evidence has been provided by the proton magnetic resonance spectrum which has two broad absorptions, at 11.22 and 7.70 ppm accounting for two hydrogens each pertaining to proton attached to -NH- and -OH present in the part of amino acid units. Methylene unit of glycine showed up at 4.18 as a singlet with integration for four hydrogens. M+1 peak in the mass spectrum with reasonable intensity gave evidence for the molecular weight of the compound. The fragmentation pattern observed is in accordance with the structure proposed.

The products (**6a-j**) have been found to possess very good antibacterial properties **(Table 1).** Among the tested compounds, **6e**, **6i** and **6j** exhibited maximum inhibition activity against both the bacterial strains.

CONCLUSION

A series of some new 2,2'(2,5-dimethoxy-3,6-dioxocycloxa-1,4-diene-1-4-diyl)bis(azanediyl) diacetic acid analogues **6(a-j)** was successfully synthesized by the condensation of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone **4** with different amino acids **5(a-j)** in the presence of triethylamine in ethanol. When the synthesized compounds were screened for their antibacterial activity, all the compounds showed good activity against both the bacterial strains like other 1,4-benzoquinone-amino acid conjugates. The present work turned out to be an interesting study of compounds with potential structural variations and promising physiological activities for further studies *in vivo* and *in vitro* conditions. Further investigations are expected to attract the attention of both chemists and biologists in pursuit of active drug development.

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