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An efficient synthesis and biological activity of substituted *p*-benzoquinones☆

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Abstract—An efficient synthesis of 2,5-diarylamino-3,6-dichloro-1,4-benzoquinone derivatives has been achieved by condensing mono substituted anilines with tetrachloro-*p*-benzoquinone in presence of fused sodium acetate as condensing agent under microwave irradiation without any solvent. All the synthesized compounds were tested for their antibacterial and antitumour activity using standard drugs.

1. Introduction

Quinones make up a large class of compounds with diverse biological activity. They can be found in many animal and plant cells and are widely used as anticancer, antibacterial or antimalarial drugs as well as fungicides.[1,2] Quinones are involved in various bioenergetic processes as important transport agents. These compounds have also attracted considerable attention because of their biological activity and chemotherapeutic value. Various quinones containing oxygenated aromatic rings have been reported to present biological activities.[3–5]

A variety of 1,4-benzoquinones and their nitrogen analogues have been reported for their antitumour activities.[6–8] Quinones that act against animal tumours are thought to function as bio-reductive alkylating agents.[9–11]

They play important role in biological functions including a role in oxidative phosphorylation and electron transfer.[12,13]

Microwave irradiation is well known to promote the synthesis of a variety of compounds, where chemical reactions are accelerated because of selective absorption of microwave by polar molecules.[14] As a part of our programme towards the non traditional approach to the experimental setup of organic reactions, the concept of 'microwave induced organic reaction enhancement' (MORE) chemistry has been utilized for rapid, sustainable and efficient synthesis. Microwave-assisted organic synthesis has attracted attention in recent years due to enhanced reaction rates,high yields, improved purity, ease of work up after the reaction and ecofriendly reaction conditions compared to the conventional methods.

The present work reveals the comparative aspects of synthesis of substituted-*p*-benzoquinones (conventional and microwave) and their characterization. The compounds were tested for AST (Antimicrobial Susceptibility Test) against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 10536) and *Pseudomonas aeruginosa* (ATCC 25619) using Kirby-Bauers' disc diffusion method.[15] The synthesized compounds were studied for in vitro antitumour screening by multidrug resistance on mouse lymphoma cells transfected with human mdrl gene. The cell line was L-5178 mouse T-cell lymphoma cell line, which was infected with the pHa MDR-1/A retrovirus.[16]

2. Results and discussion

2.1. Comparative profile of synthesis

2,5-Diarylamino-3,6-dichloro-1,4-benzoquinone derivatives (**3**) were prepared by condensing mono substituted

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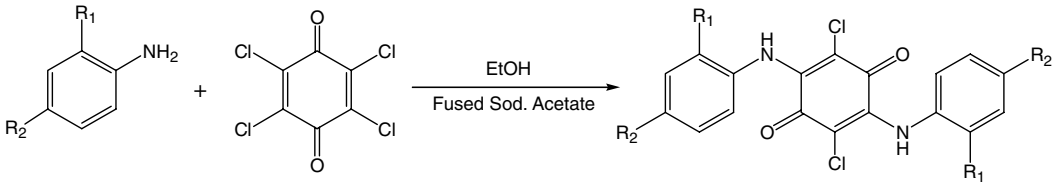


Substituents	a	b	c	d	e	f	g	h	i
R_1 =	H	CH_3	OCH_3	F	H	H	H	H	H
R_2 =	H	H	H	H	Cl	Br	CH_3	OCH_3	F

Scheme 1. Synthesis of 2,5-diarylamino-3,6-dichloro-1,4-benzoquinones (**3a**–**i**).

anilines (**1**) (2 mol) with tetrachloro-*p*-benzoquinone (**2**) (1 mole) in ethanolic medium in presence of fused sodium acetate using methods reported earlier[17] (Scheme 1).

In view of long reaction time, moderate yields (Table 1), tedious work up after the reaction and requirement of large quantity of solvent associated with conventional method, a relatively more versatile yet simplified procedure was perceived, in which substituted anilines (**1**) and tetrachloro-*p*-benzoquinone (**2**) could be made to react in the presence of fused sodium acetate under microwave irradiation without using any solvent. Microwave synthesis has received attention as a new strategy for organic synthesis due to the fact that many reactions seem to proceed with much alacrity under such conditions as opposed to the corresponding thermal-assisted reactions.[18] The strategy worked well affording the desired product in improved yields in significantly lower reaction time (Table 1). The result obtained under microwave irradiation was extrapolated to conventional heating. Reactions mentioned in Table 1 were examined by simply heating in a preheated oil-bath under the same conditions (time, temperature, pressure and medium) as mentioned in the typical procedure with microwave irradiation. It was found that no reaction occurred and that the reactants remain unchanged, even on extended time (4 h),thus demonstrating that the effect of microwave is not simply thermal.[19] The rate acceleration under microwave irradiation was due to specific microwave effect.[20] Under microwave irradiation enhanced dipole–dipole interactions between the activated reaction intermediates caused the instantaneous condensation of reactants to afford substituted-1,4-benzoquinones (**3**) without the use of any solvent in a very short time.

After analysis of the results summarized in Table 1, we found the reaction of substituted anilines (**1**) and tetrachloro-*p*-benzoquinone (**2**) in the presence of fused sodium acetate afforded **3** as needles of various colours. The elements (C, H, and N) found for all the molecular formulas (**3a**–**i**) were in close agreement with the calculated one, the UV spectrum of **3** showed a broad unstructured band at about 400 nm characteristic of *p*-benzoquinones.The IR spectrum of **3** exhibited a strong absorption band due to NH stretching at 3240 cm^{-1} and CO stretching band at 1650 cm^{-1}in addition to other bands. The ^1H NMR spectrum of **3** revealed characteristic signals for methyl protons at δ 2.28–2.42 (s, 6H, $2CH_3$), methoxy protons at δ 3.87 (s, 6H, $2OCH_3$), aromatic protons in δ 6.8–7.5 (m, 8–10H, ArH) and NH proton

Table 1. Comparative study for the synthesis of 2,5-diarylamino-3,6-dichloro-1,4-benzoquinones (**3a–i**)

Compound	Conventional method			Microwave irradiation[a]			Conventional heating[d]	
	Time (h)	Temp (°C)	Yield (%)	Time (min)	Temp[b] (°C)	Yield[c] (%)	Time[e] (h)	Yield (%)
3a	4	reflux	70	1.5	68	90	4	Nil
3b	4	reflux	65	1.5	72	86	4	Nil
3c	4	reflux	62	1.5	70	84	4	Nil
3d	4	reflux	60	2.0	69	83	4	Nil
3e	4	reflux	86	1.5	73	90	4	Nil
3f	4	reflux	75	2.0	67	85	4	Nil
3g	4	reflux	75	2.0	66	85	4	Nil
3h	4	reflux	80	1.5	68	89	4	Nil
3i	4	reflux	70	2.5	65	83	4	Nil

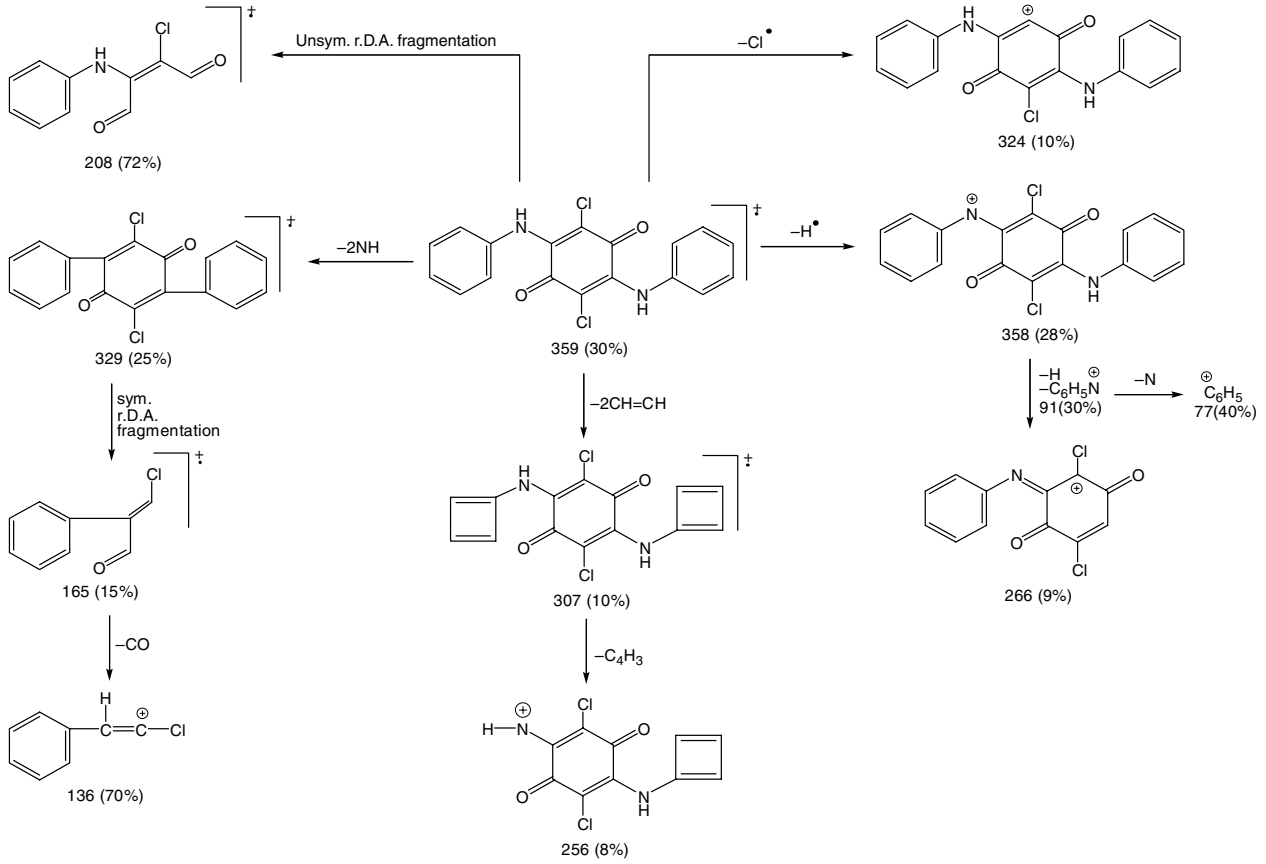
[a] Reaction mixture in microwave oven was irradiated at power output of 160 W.
[b] The final internal temperature of the reaction mixture was measured by non-contact IR thermometer.
[c] Isolated yield of purified compounds that exhibited physical and spectral properties in accordance with assigned structure.
[d] Conventional heating was conducted in thermally preheated oil-bath under identical reaction conditions and temperature used under microwave.
[e] Extended reaction time for reaction in thermally preheated oil-bath under identical reaction conditions and temperature used under microwave.

at δ 8.30 (s, 1H, NH, exchanged with deuterium). Formation of the **3** was further confirmed on the basis of ^{13}C NMR spectroscopy and mass spectroscopy. In the ^{13}C NMR spectrum of **3**, sharp signals were observed at 17.6 (CH_3), 55.9 (OCH_3), 110.6–152.2 (aromatic ring carbons), 172.5 (C=O).The FAB MS of **3a** (Scheme 2) showed the molecular ion peak at *m/z* 359 (30%, M^+) and also isotopic abundance peak at *m/z* 361 (16%, M^++2). Molecular ion (M^+) after losing one chlorine atom produces a fragment at *m/z* 324 (10%, M^+-Cl) along with isotopic abundance at *m/z* 326 (6.5%, M^++2−Cl). All the fragments containing two chlorine atoms 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, 91and 77 supports the presence of two NH groups positioned in-between two aromatic side rings and central ring of **3a**. Fragment 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 (70%, 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 the 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** along with other spectral studies clearly suggests that two chlorine and two keto groups are attached to central ring at 3,6 and 2,5 positions, respectively, while the two anilino (C_6H_5NH) groups are attached to the central ring at the remaining 1,4 positions. Similarly mass spectrum of **3b** (Scheme 3) also support the proposed structure of the compound.

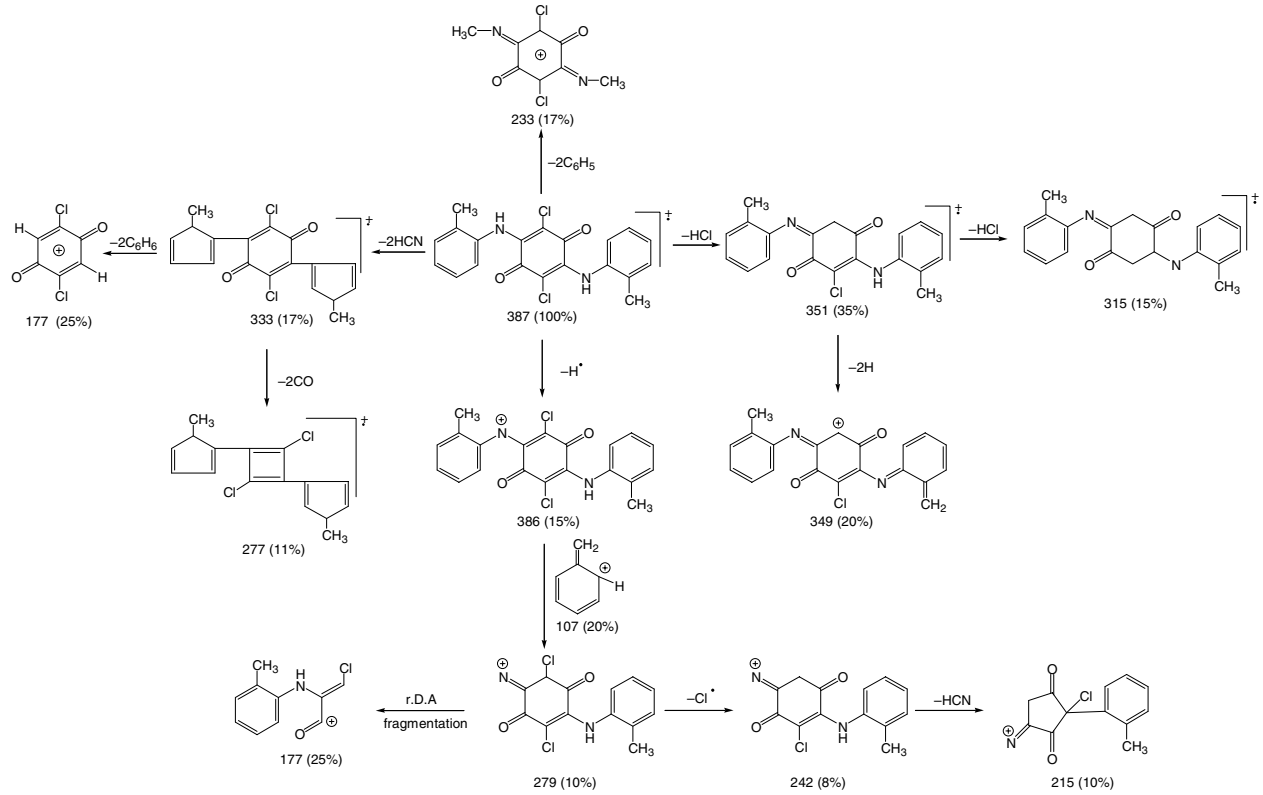
Thus on the basis of spectral data all products **3a**–**i** have been identified.

2.2. Antibacterial screening

As we see from Table 2 compound **3c** was found active against all the strains of bacteria under study (above 50% as compared to the standard drug Gentamycine). Compound **3e** show maximum activity against *S. aureus* while compound **3b** exhibits minimum against it. Against *E. coli* maximum activity is exhibited by compound **3f** and minimum by compound **3a**. While against



Scheme 2. Mass fragmentation pattern of 2,5-dianilino-3,6-dichloro-1,4-benzoquinone (**3a**).



Scheme 3. Mass fragmentation pattern of 2,5-bis(2′-methylanilino)-3,6-dichloro-1,4-benzoquinone (**3b**).

Table 2. Zone of inhibition for antibacterial activity of 2,5-dianilino-3,6-dichloro-1,4-benzoquinone

Compound	Diameter of zone of inhibition					
	S. aureus ATCC 25923		*E. coli* ATCC 10536		*P. aeruginosa* ATCC 25619	
	Mean	%[a]	Mean	%[a]	Mean	%[a]
3a	08.53 ± 0.57	11.68	08.55 ± 0.33	14.07	09.11 ± 0.87	15.46
3b	08.50 ± 0.32	11.55	09.11 ± 0.74	17.16	09.89 ± 0.32	19.33
3c	17.75 ± 0.87	54.27	15.34 ± 0.77	51.55	16.42 ± 0.65	51.78
3d	12.33 ± 0.66	29.24	13.45 ± 0.00	41.11	13.21 ± 0.62	35.83
3e	18.13 ± 0.88	56.03	13.86 ± 0.43	43.38	15.85 ± 0.76	48.96
3f	13.52 ± 0.43	34.73	16.75 ± 0.55	59.32	18.88 ± 0.33	64.02
3g	12.62 ± 0.84	30.58	11.98 ± 0.49	33.00	14.08 ± 0.44	40.16
3h	16.18 ± 0.45	47.02	12.11 ± 0.49	33.71	14.70 ± 0.31	43.24
3i	12.45 ± 0.68	29.79	14.18 ± 0.45	45.14	16.23 ± 0.22	50.84
Gent	27.65 ± 1.47	100	24.12 ± 0.54	100	26.12 ± 0.97	100
DMF	06.00 ± 0.00	00	06.00 ± 0.00	00	06.00 ± 0.00	00

Gent, gentamycine.
Mean, mean value of diameter of inhibition zone with standard error in millimeters.
[a] Percentage was calculated after subtracting disc diameter (6 mm) from all observations.

P. aeruginosa antibacterial action of compound **3f** is maximum. Other compounds **3c** and **3i** also show good inhibition (above 50%) against *P. aeruginosa*. Minimum activity against this strain of bacteria is shown by compound **3a**. Based on the above finding it is very difficult to draw correlation between structures and activity (Table 2).

2.3. Antitumour screening

In the present investigation, *p*-benzoquinone compounds were studied for reversal of multi drug resistance on mouse lymphoma cells transfected with human mdrl gene. As we see from Table 3 compound **3a** is very effective because its fluorescence activity ratio is comparatively higher than other compounds and verapamil, although compound **3c** has some marginal effects. But compounds **3b** and **3e** do not affect cell size, but the cytoplasmic granulation was enhanced.

3. Conclusion

We have developed an economical, solvent free, very efficient microwave-assisted protocol for the synthesis of 2,5-dianilino-3,6-dichloro-1,4-benzoquinone which

Table 3. Flourescence activity ratio of 2,5-dianilino-3,6-dichloro,1,4-benzoquinone

Compound	Concentration (μg/ml)	FSC	SSC	FL-1	Flourescence activity ratio
PAR control		409.25	100.33	1135.85	
MDR control		425.01	120.64	35.73	
VERAPAMIL	5	420.89	117.21	183.97	5.15
DMSO	20	467.44	149.20	19.12	0.54
3a	20	346.50	142.65	218.02	6.10
3b	20	457.14	226.44	19.03	0.53
3c	20	363.44	161.20	58.26	1.63
3e	20	405.29	138.08	17.17	0.48

can be a viable alternative to the conventional synthesis. In all cases, a comparison of the reactions using either thermal or microwave heating under the same conditions shows clearly a specific (non-thermal) microwave effect.

The antibacterial activity in terms of zone of inhibition is exhibited by all the compounds against all the strains of bacteria under study (*S. aureus*, *E. coli* and *P. aeruginosa*). Maximum antibacterial activity is shown against *P. aeruginosa* by the compound **3f**. In the in vitro antitumour screening compound **3a** was found to be very effective while compound **3c** was marginally effective. There is no such permanent structure–activity relationship between the design of molecule under study and its biological activity.

4. Experimental

4.1. Equipment

Reagent-grade chemicals were used without further purification. The substrates and solvents were used as received. All the melting points are uncorrected. The purity of synthesized compounds was checked by TLC studies. UV/vis spectra were recorded in Perkin-Elmer Lamda 15 UV/vis spectrophotometer. IR spectra were measured on FT IR Perkin-Elmer (Spectrum RX1) spectrometer (ν_{max} in cm^{-1}) using KBr disc. ^1H NMR and ^{13}C NMR were recorded in DMSO with TMS as the internal standard at 300 MHz on a Bruker DRX-300 spectrometer. Fast atom bombardment mass spectra (FAB MS) were recorded at room temperature on a Jeol SX-102/DA-6000 Mass spectrometer/Data System using Aragon/Xenon (6 kV,10 mA) as the FAB gas. The accelerating voltage was 10 kV. C, H and N elements were analyzed by Elementar Vario EL III Carlo Erba 1108 elemental analyzer. Reactions under microwave irradiations were carried out in microwave oven model LG MS-194W operating at 160 W generating 2450 MHz frequency. Internal temperature of reaction mixture was measured on Mini Gun Type Non-Contact IR thermometer, Model No. 8868.

4.2. Investigation of the synthesis of 2,5-diarylamino-3,6-dichloro-1,4-benzoquinones (3a–i)

4.2.1. Under conventional condition. (a) To a stirred suspension of (0.01 mol) tetrachloro-*p*-benzoquinone(**2**) and (0.01 mol) anhydrous sodium acetate in ethanol (100 ml) was added an alcoholic solution of (0.02 mol) mono substituted aryl amines (**1a–i**). The resultant mixture was refluxed for 4 h. The reaction was hot filtered and residue was first washed with hot water and finally with 30% ethanol to give the desired products. Products were recryztallised from acetone/benzene to give 2,5-diarylamino-3,6-dichloro-1,4-benzoquinones (**3a–i**)[17] (Scheme 1).

(b) The synthesis of title compounds (**3a–i**) was attempted by condensing (2 mmol) mono substituted anilines (**1a–i**) with (1 mmol) tetrachloro-*p*-benzoquinones (**2**) in presence of (1 mmol) anhydrous sodium acetate using a preheated oil-bath under near identical conditions of time, temperature, pressure and medium as under microwave. TLC indicated the unchanged reactants for heating up to 2.5 min. Even extended heating for 4 h did not lead to formation of any product.

4.2.2. Under microwave irradiation. The title compounds (**3a–i**) were synthesized by condensing (2 mmol) mono substituted anilines (**1a–i**) with (1 mmol) tetrachloro-*p*-benzoquinones (**2**) in presence of (1 mmol) anhydrous sodium acetate. The reaction was carried out in Erlenmeyer flask capped with a funnel under microwave irradiation at 160 W in microwave oven, at the marked hot spots, for the specific period of time. The completion of the reaction was monitored by silica gel TLC. (Benzene/acetone 60:40). To ensure the reproducibility every reaction was carried out five times in microwave. The final temperature of the reaction mixture was measured by non-contact IR thermometer specifically fitted for measuring the internal temperature. After cooling the residue was washed with hot water and 30% ethanol to give **3a–i**.

4.3. Spectral data of synthesized compounds (3a–i)

4.3.1. 2,5-Dianilino-3,6-dichloro-1,4-benzoquinone (3a). Yield: 323 mg, 90%; as yellowish green needles, mp 248 °C (from dimethylformamide/benzene); Anal. Calcd for $C_{18}H_{12}Cl_2N_2O_2$: C, 60.16; H, 3.34; N, 7.79. Found: C, 60.13; H, 3.31; N, 7.72%. UV/vis λ_{max} (cm^{-1}) 388; ν_{max} (cm^{-1}) (KBr) 3239 (N–H), 1651 (C=O), 1571, 1353, 1248, 1194, 1111, 1026; δ_H (300 MHz; DMSO) 8.302 (1H, s, NH, D_2O exchangeable) 7.125–7.401 (10H, m, ArH (2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″ and 6″)); δ_c (300 MHz; DMSO) 124.8, 125.3, 127.9, 172.9 (C=O); FAB MS (298 °C) *m/z* (%, fragment) 359 (30%, M^+), 361 (16%, M^++2), 358 (28%, M^+−H) 329 (25%, M^+−2NH), 324 (10%, M^+−Cl), 326 (6.5%,

M^++2−Cl), 307 (10%, $M^+-2C_2H_2$), 266 (9%, M^+−H–C_6H_5N), 208 (72%, unsym. rDA frag.), 165 (15%, sym. rDA frag.), 136 (70%, rDA frag.-CO), 91 (30%, $C_6H_5N^+$), 77 (40%, $C_6H_5^+$).

4.3.2. 2,5-Bis (2′-methylanilino)-3,6-dichloro-1,4-benzoquinone (3b). Yield: 333 mg, 86%; as brown needles mp 198 °C (from dimethylformamide/benzene); Anal. Calcd for $C_{20}H_{16}Cl_2N_2O_2$: C, 61.97; H, 4.16; N, 7.23; C, 60.13; H, 3.31; N, 7.72. Found: C, 61.92; H, 4.12; N, 7.19%. UV/vis λ_{max} (cm^{-1}) 367.2; ν_{max} in cm^{-1} (KBr) 3242 (N–H), 1652 (C=O), 1564, 1328, 1236, 1195, 1112, 1022; δ_H (300 MHz; DMSO) 8.192 (1H, s, NH, D_2O exchangeable), 7.235–7.260 (2H, d, J = 7.5 Hz, ArH (6′ and 6″)), 7.181–7.212 (4H, dd, J = 4.5 Hz, J = 4.8 Hz, ArH (4′, 5′, 4″ and 5″)) 7.086–7.107 (2H, d, J = 6.3 Hz, ArH (3′ and 3″)), 2.280 (6H, s, $2CH_3$); δ_c (300 MHz; DMSO) 17.6 (CH_3), 125.2, 126.9, 127.8, 129.5, 134.6, 137.6, 139.5, 144.7, 172.5 (C=O) FAB MS (298 °C) *m/z* (%, fragment), 387 (100, M^+), 389 (33, M^++2), 386 (15, M^+−1), 351 (35, M^+−HCl), 315 (15, M^+−2HCl), 307 (48), 289 (25), 279 (10), 277 (11), 242 (8), 233 (17, M^+−HCN), 215 (10), 203 (15), 191 (8), 177 (25), 107 (20).

4.3.3. 2,5-Bis(2′-methoxyanilino)-3,6-dichloro-1,4-benzoquinone (3c). Yield: 352 mg, 84%; as green needles mp 283 °C (from dimethylformamide/benzene); Anal. Calcd for $C_{20}H_{16}Cl_2N_2O_4$: C, 57.29; H, 3.84; N, 6.68. Found: C, 57.26; H, 3.81; N, 6.65%. UV/vis λ_{max} (cm^{-1}) 390; ν_{max} in cm^{-1} (KBr) 3240 (N–H), 1660 (C=O), 1580, 1340, 1200, 1140, 1040; δ_H (300 MHz; DMSO) 8.240 (1H, s, NH, D_2O exchangeable), 7.221–7.246 (2H, dd, J = 1.5 Hz, J = 4.2 Hz, ArH (3′ and 3″)), 7.050–7.076 (2H, dd, J = 6.6 Hz, J = 1.2 Hz, ArH (6′ and 6″)), 6.902–6.984 (4H, m, ArH (4′, 5′, 4″ and 5″)), 3.87 (6H, s, $2OCH_3$); δ_c (300 MHz; DMSO) 55.9 (OCH_3), 110.6, 119.6, 125.8, 126.9, 127.8, 139.7, 143.5, 152.2, 172.8 (C=O); FAB MS (298 °C) *m/z* (%, fragment) 419 (100, M^+), 421 (33, M^++2), 418 (50, M^+−1), 388 (25, M^+-OCH_3), 387 (20, M^+-CH_3OH), 357 (38, M^+-2OCH_3), 336 (25), 315 (5), 273 (12), 260 (18), 245 (8) 228 (11), 209 (12), 178 (10), 152 (20), 120 (35), 107 (60), 76 (20).

4.3.4. 2,5-Bis(2′-fluoroanilino)-3,6-dichloro-1,4-benzoquinone (3d). Yield: 328 mg, 83%; as brown needle mp 318 °C (from dimethylformamide/benzene); Anal. Calcd for $C_{18}H_{10}F_2Cl_2N_2O_2$: C, 54.71; H, 2.55; N, 7.08. Found: C, 54.70; H, 2.52; N, 7.05%. UV/vis λ_{max} (cm^{-1}) 360; ν_{max} in cm^{-1} (KBr) 3240 (N–H), 1656 (C=O), 1576, 1331, 1221, 1105, 1035; δ_H (300 MHz; DMSO) 8.280 (1H, s, NH, D_2O exchangeable), 7.548–7.646 (2H, dd, J = 1.5 Hz, J = 7.5 Hz, ArH (3′ and 3″)), 7.374–7.480 (2H, dd, J=1.2 Hz, J = 6.8 Hz, ArH (6′ and 6″)), 7.210–7.327 (4H, m, ArH (4′, 5′, 4″ and 5′)); δ_c (300 MHz; DMSO) 126.6, 127.2, 128.2, 128.8, 133.8, 137.7, 139.6, 144.4, 162.1, 171.2 (C=O); FAB MS (298 °C) *m/z* (%, fragment), 395 (48, M^+), 397 (16, M^++2), 394 (15, M^+−1), 376 (10), 370 (9), 365 (38, M^+−2NH), 361 (17, M^+−2F), 329 (50), 317 (10), 307 (20), 289 (20), 273 (7), 254 (70), 232 (40), 228 (9), 214 (10), 176 (90), 154 (89), 136 (85), 101 (80), 89 (35).

4.3.5. 2,5-Bis(4′-chloroanilino)-3,6-dichloro-1,4-benzoquinone (3e). Yield: 385 mg, 90%; as dark green needles mp 350 °C (from dimethylformamide/benzene); Anal. Calcd for $C_{18}H_{10}Cl_4N_2O_2$: C, 50.46; H, 2.33; N, 6.54. Found: C, 50.42; H, 2.31; N, 6.49%. UV/vis λ_{max} (cm^{-1}) 385; ν_{max} in cm^{-1} (KBr) 3230 (N–H), 1650 (C=O), 1590, 1322, 1250, 1190, 1045; δ_H (300 MHz; DMSO) 8.26 (1H, s, NH, D_2O exchangeable), 7.505–7.534 (2H, d, J = 8.7 Hz, ArH (3′ and 3″)), 7.375–7.404 (2H, d, J = 8.7 Hz, ArH (5′ and 5″)), 7.082–7.111 (2H, d, J = 8.7 Hz, ArH (2′ and 2″)), 7.033–7.062 (2H, d, J = 8.7 Hz, ArH (6′ and 6″)); δ_c (300 MHz; DMSO) 124.6, 125.2, 127.2, 128.9, 134.8, 136.7, 140.6, 145.4, 160.1, 170.2 (C=O); FAB MS (298 °C) *m/z* (%, fragment), 428 (77, M^+), 430 (100, M^++2), 432 (44, M^++4), 434 (10, M^++4), 427 (25, M^+−1), 357 (40, M^+−2Cl), 392 (46, M^+−HCl), 336 (11), 321 (17), 289 (88), 265 (37), 235 (26), 205 (15), 189 (76), 157 (50) 136 (35) 105 (65), 77 (40).

4.3.6. 2,5-Bis(4′-bromoanilino)-3,6-dichloro-1,4-benzoquinone (3f). Yield: 439 mg, 85%; as yellowish-green needles mp 325 °C (D) (from dimethylformamide/benzene); Anal. Calcd for $C_{18}H_{10}Cl_2Br_2N_2O_2$: C, 41.77; H, 1.93; N, 5.41. Found: C, 41.70;H, 1.90;N, 5.36%. UV/vis λ_{max} (cm^{-1}) 390; ν_{max} in cm^{-1} (KBr) 3230 (N–H), 1650 (C=O), 1595, 1318, 1245, 1175, 1100, 1035; δ_H (300 MHz; DMSO) 8.24 (s, 1H, NH, D_2O exchangeable), 7.583–7.612 (2H, d, J = 8.7 Hz, ArH (3′ and 3″)), 7.439–7.468 (2H, d, J = 8.7 Hz, ArH (5′ and 5″)), 7.380–7.409 (2H, d, J = 8.7 Hz, ArH (2′ and 2″)), 7.089–7.118 (d, 2H, J = 8.7 Hz, ArH (6′ and 6″)); δ_c (300 MHz; DMSO) 124.3, 125.2, 127.2, 128.2, 130.2, 134.6, 137.2, 142.6, 159.8, 171.2 (C=O); FAB MS (298 °C) *m/z* (%, fragment), 517 (38, M^+), 519 (100, M^++2), 521 (89, M^++4), 523 (32, M^++4), 482 (45, M^+−Cl), 446 (85, M^+−2Cl), 437 (25, M^+−Br), 357 (40, M^+−2Br), 337 (15), 313 (26), 291 (28), 256 (84), 218 (23), 200 (17), 188 (65), 165 (45), 136 (70), 108 (43), 77 (35).

4.3.7. 2,5-Bis(4′-methylanilino)-3,6-dichloro-1,4-benzoquinone (3g). Yield: 329 mg, 85%; as reddish brown needles mp 275 °C (from dimethylformamide/benzene); Anal. Calcd for $C_{20}H_{16}Cl_2N_2O_2$: C, 62.03; H, 4.16; N, 7.23. Found: C, 62.01; H, 4.12; N, 7.19%. UV/vis λ_{max} (cm^{-1}) 420; ν_{max} in cm^{-1} (KBr) 3240 (N–H), 1650 (C=O), 1562, 1325, 1233, 1193, 1111, 1020; δ_H (300 MHz; DMSO) 8.323 (1H, s, NH, D_2O exchangeable) 7.158–7.185 (4H, d, J = 8.1 Hz, ArH (2′, 6′, 2″ and 6″)), 7.013–7.040 (4H, d, J = 8.1 Hz, ArH (3′, 5′, 3″ and 5″)), 2.42 (6H, s, $2CH_3$); δ_c (300 MHz; DMSO) 17.9 (CH_3), 125.6, 127.2, 127.5, 129.9, 134.8, 137.1, 144.6, 170.2 (C=O); FAB MS (298 °C) *m/z* (%, fragment), 387 (100, M^+), 389 (63, M^++2), 391 (10, M^++4), 386 (72, M^+−H), 351 (25, M^+−HCl), 349 (10), 333 (11) 315 (10, M^+−2HCl), 307 (45), 279 (10) 277 (14), 242 (18), 233 (15), 215 (8), 203 (15), 191 (10), 177 (25), 154 (80), 152 (24), 136 (62), 135 (15), 107 (20).

4.3.8. 2,5-Bis(4′-methoxyanilino)-3,6-dichloro-1,4-benzoquinone (3h). Yield: 373 mg, 89%; as dark brown needles mp 240 °C (from dimethylformamide/benzene); Anal.

Calcd for $C_{20}H_{16}Cl_2N_2O_4$: C, 57.29; H, 6.65; N, 6.68. Found: C, 57.25; H, 6.60; N, 6.16%. UV/vis λ_{max} (cm^{-1}) 400; ν_{max} in cm^{-1} (KBr) 3239 (N–H), 1654 (C=O), 1575, 1337, 1251, 1195, 1138, 1040; δ_H (300 MHz; DMSO) 8.320 (1H, s, NH, D_2O exchangeable) 7.060–7.090 (4H, d, $J = 9.0$ Hz, ArH (3′, 5′, 3″ and 5″)), 6.870–6.900 (4H, d, $J = 9.0$ Hz, ArH (2′, 6′, 2″ and 6″)), 3.88 (6H, s, $2OCH_3$); δ_c (300 MHz; DMSO) 55.7, 111.1, 119.7, 126.6, 127, 127.9, 136.5, 143.6, 153.8, 172.8 (C=O); FAB MS (298 °C) *m/z* (%, fragment), 419 (83, M^+), 421 (49, M^++2), 418 (50, M^+−1), 413 (10), 388 (18, M^+-OCH_3), 387 (12, M^+-CH_3OH), 356 (9, M^+-2OCH_3), 353 (10), 336 (5), 315 (8), 273 (13), 260 (10), 245 (9), 228 (8), 215 (7), 209 (12), 183 (9), 178 (12), 120 (48), 107 (56), 88 (45), 76 (30), 72 (20).

4.3.9. 2,5-Bis(4′-fluoroanilino)-3,6-dichloro-1,4-benzoquinone (3i). Yield: 327 mg, 83%; as green needles mp260 °C (from dimethylformamide/benzene); Anal. Calcd for $C_{18}H_{10}F_2Cl_2N_2O_2$: C, 54.68; H, 6.65; N, 4.08. Found: C, 54.63;H, 6.60;N, 4.05%. UV/vis λ_{max} (cm^{-1}) 390; ν_{max} in cm^{-1} (KBr) 3239 (N–H), 1651 (C=O), 1573, 1326, 1216, 1151, 1099, 1031; δ_H (300 MHz; DMSO) 8.33 (1H, s, NH, D_2O exchangeable) 7.175–7.200 (4H, d, $J = 7.5$ Hz, ArH (3′, 5′, 3″ and 5″)), 7.137–7.169 (4H, d, $J = 6.9$ Hz, ArH (2′, 6′, 2″ and 6″)); δ_c (300 MHz; DMSO) 123.5, 126.7, 128.2, 128.9, 135.2, 138.7, 140.2, 145.2, 170.8 (C=O); FAB MS (298 °C) *m/z* (%, fragment), 395 (63, M^+), 397 (40, M^++2), 394 (45, M^+−1), 370 (10), 365 (15, M^+−2NH), 361 (56, M^+−2F), 317 (8), 289 (15), 273 (6), 254 (62), 232 (38), 198 (8), 176 (85), 165 (11), 154 (82), 136 (80), 120 (18), 101 (90), 89 (32).

4.4. Antibacterial screening

The Antimicrobial Susceptibility Testing (AST) was accomplished by the Kirby-Bauers' disc diffusion method. All the synthesized compounds were screened for their antibacterial activity against *S. aureus* (ATCC 25923), *E. coli* (ATCC 10536) and *P. aeruginosa* (ATCC 25619) using Muller Hinton Agar media (Hi Media). The sample solution was prepared by dissolving 10 μg of each of the compound in 1.0 ml of DMF. Gentamycine (10 mcg) was used as standard drug for comparison. The sterilized Whatman filter paper discs of approximately 6 (No. 1) mm were dipped in sample solution and dried in oven. These discs were placed on the medium previously seeded with the organisms in petri dishes at suitable distances. The petri dishes were stored in an incubator at 30 ± 2 °C for 24 h. The zone of inhibition thus formed around each disc containing the test compound was measured accurately in millimeters.

4.5. Antitumour screening

All the compounds were studied for in vitro antitumour screening. The compounds were screened for reversal of multidrug resistance on mouse lymphoma cells transfected with human mdrl gene. The cell line used was L-5178 mouse T-cell lymphoma cell line which was infected with the pH a MDR-1/A retrovirus.

Peripheral human blood (PBL) samples were obtained from volunteer cancer patients and PBL was prepared by Ficoll-Hypaque density gradient centrifugation. The sensitive leukaemia cell lines L5178 and its MDR1 gene transfected resistant pair were obtained. MDR expressing cells were cultured in the presence of colchine up to 48 h before being used in the drug uptake assay.

4.5.1. Cell and fluorescence uptake, mdrl reversal effect. The L5178 mouse T lymphoma cell line was infected with the pHa MDR1/A retrovirus. MDR1 expressing cell lines were selected by culturing the infected cells with 60 mg/ml colchine to maintain expression of the MDR phenotype. The L5178 MDR cell line and the L5178Y parent cell line were grown in McCoy's 5A medium with 10% heat-inactivated horse serum, L-glutamine and antibiotics. The cells were adjusted to a concentration of 2×10^6/ml and resuspended in serum free McCoy's 5A medium and the cells were distributed into 0.5 ml aliquot to Eppendorf centrifuge tubes. Then the tested compounds were added in 20 μg/ml of the 1.0 mg/ml stock solutions and the samples were incubated for 10 min at room temperature. Then 10 μl (5.2 μM final concentration) indicator Rhodamine 123 was added to the samples and the cells were incubated for further 20 min at 37 °C, washed twice and resuspended in 0.5 ml phosphate-buffered saline (PBS) for analysis. The fluorescence of cell population was measured by flow cytometry using Becton–Dickinson FACScan instrument. Verapamil has been used as a positive control in the Rhodamine 123 exclusion experiments, Epstein and Shafron calculated for parental and mdr cell lines as compared to untreated cells. An activity ratio was calculated by the following equation on the basis of measured fluorescence values.

The results were analyzed by using cell quest software (Becton–Dickinson) and presented as arbitrary units of size (FSC) and granularity (SSC) and as the average fluorescence intensity (FL-1).

$$R = \frac{\text{Mdr treated/Mdr control}}{\text{parental treated/parentel control}}$$

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