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BIOLOGICAL ACTIVITIES OF SOME SUBSTITUTED–1, 4–BENZOQUINONES

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ABSTRACT

Symmetrically substituted 2,5–diarylamino–3,6–dihalo–1,4–benzoquinone were prepared by the condensation of aryl amine with chloranil/bromanil in the molar ratio of 2 : 1. The compounds were studied for *in vitro* antitumour screening by multidrug resistance on mouse lymphoma cells transfected with human mdrl gene. Compunds were also tested for their antibacterial activity against *Pseudomonas solanaceacum* (*P. sola*), *Pseudomonas syringae* (*P. syr*) and *Pseudomonas viridiflave* (*P. viri*) using filter paper disc method.

Key words: 1,4–Benzoquinones, Biological activity, Antitumour activity

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 those act against animal tumours are thought to function as bioreductive alkylating agents[9–11]. They play important role in biological functions including a role in oxidative phosphorylation and electron transfer[12,13].

Here, activities of some 2,5–diarylamino–3,6–dihalo–1,4–benzouinone derivatives have been reported as antitumour and antibacterials.

The title compounds (**3**) were prepared by condensing mono– or disubstituted anilines (**1**) (2 mole) with halogeno–p–benzoquinone (**2**) (chloranil or bromanil, 1 mole) in ethanolic medium in presence of fused sodium acetate using methods reported earlier[14] (Scheme–1) (Table 1).

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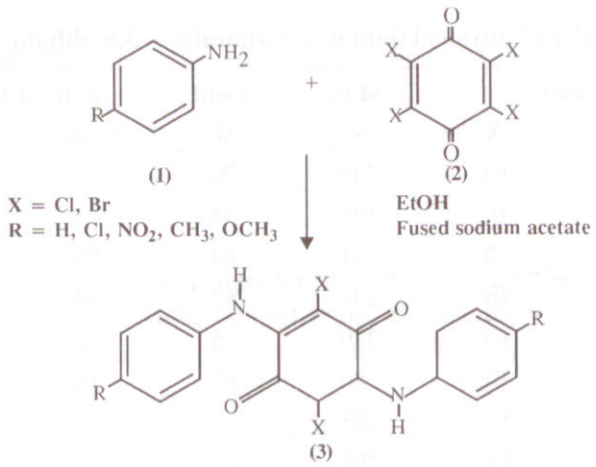

The compounds were tested for antibacterial activity against *Pseudomonas solanaceacum* (*P. sola*), *Pseudomonas syringae* (*P. syr*) and *Pseudomonas viridiflave* (*P. viri*) using filter paper disc method[15] and were studied for *in vitro* antitumour screening by multidrug resistance on mouse lymphoma 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[16,17].

Antibacterial screening

The activity against bacteria was evaluated by the paper disc plate method. For this purpose, pure cultures of the organisms were dissolved in peptone water (1 : 1) and then uniformly seeded on the nutrient agar plates having the composition; Peptone 10 g, yeast extract 3 g, sodium chloride 3 g, beef extract 3 g, glucose 1 g, distilled water 100 mL, agar agar 1 g with pH 6.8–7.2. The sample solution was prepared by dissolving 10 µg of each of the compound in 1.0 mL of DMF. The sterilized Whatman filter paper disc 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 distance. The petri dishes were stored in an incubator at 30 ± 2°C for 24 hrs. The zone of inhibition thus formed around each disc containing the test compound was measured accurately in mm. The bacterial organisms used in the present investigation were *Pseudomonas solanaceacum* (*P. sola*), *Pseudomonas syringae* (*P. syr*) and *Pseudomonas viridiflave* (*P. viri*). Under similar conditions, control experiment was carried out using ampicillin, genstar and streptomycin as a standard for comparison.

Compounds (**3–b. f, g and h**) were more active against *P. syr*, while other compounds found to have less moderate activity than the ampicillin, genstar and streptomycin. Compounds (**3–c, d, f and h**) were found more active against *P. sola*, while other compounds found to have less moderate activity than the ampicillin, genstar and streptomycin. Compounds (**3–a, c, f, g and i**) were found more active against *P. viri*, while other compounds found to have less moderate activity than the ampicillin, genstar and streptomycin.

Table 1. Antibacterial and physical data of 2,5–dianilino–3,6–dihalo, 1,4–benzoquinone

	Compound		M.P.	Yield	Zone of Inhibition (in mm)		
	R	X	°C	%	*P. syr.*	*P. sola.*	*P. viri*
3a.	H	Cl	248	70	12	6	14
3b.	H	Br	199	75	14	12	8
3c.	Cl	Cl	201	68	10	15	12
3d.	Cl	Br	244	62	10	14	7
3e.	NO_2	Cl	320	65	8	6	8
3f.	NO_2	Br	280	65	14	14	12
3g.	OCH_3	Cl	283	62	15	12	14
3h.	OCH_3	Br	295	65	16	14	8
3i.	CH_3	Cl	198	65	12	6	14
3j.	CH_3	Br	219	75	8	6	6
	Ampicillin				13	11	15
	Genstar				9	8	8
	Streptomycin				15	13	16

Antitumour screening

The screening of compunds was carried out in the laboratory at Szeged Foundation for Cancer Research, University of Szeged, Domter 10, Hungary. 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 centriguation. The sensitive leukemia 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 hours before being used in the drug uptake assay.

Cell and fluorescence uptake, mdr reversal effect

The L5178 mouse T cell 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 experssion of the MDR phenotype. The L5178 MDR cell line and 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 x 10^6/mL and resuspended in serum free McCoy's 5A medium and the cells were distributed into 0.5 mL aliquote 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 (Hickman *et al.*, 1985). Epstein and Shafron calculated for parental and mdr cell lines as compared to untreated cells. An activity ratio was calcualated by the following equation (Hickman *et al.*, 1985) on the basis of measured fluorescence values.

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

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

In the present investigation, p–benzoquinone compounds were studied for reversal of multidrug resistance on mouse lymphoma cells transfected with human mdrl gene. As it is clear from Table 2, compounds **(3 a, b and f)** are very effective because its fluorescence activity ratio

Table 2. Fluorescence activity ratio of some *p*–benzoquinone derivatives

Compound	Conc. µg/mL	FSC	SSC	FL–1	Fluorescence 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	346.5	142.65	218.02	6.10
3b.	20	345.94	137.16	389.30	10.90
3c.	20	405.29	138.08	17.17	0.48
3d.	20	395.74	136.31	13.24	0.37
3e.	20	250.67	122.00	103.76	2.90
3f.	20	264.11	125.08	212.12	5.94
3g.	20	363.44	161.20	58.26	1.63
3h.	20	367.95	166.46	39.76	1.11
3i.	20	457.14	226.44	19.03	0.53
3j.	20	465.19	223.24	46.30	1.30

is comparatively higher than other compounds and verapamil, although compounds (**3 e, g, h and j**) have some marginal effects. But compuonds (**3 c, d and i**) do not afffect cell size, but the cytoplasmic granulation was enhanced.

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