Synthesis and Pharmacological Studies of Some (1,4)-Naphthoquinono[3,2-c]-1H-pyrazoles, 2-Substituted Amino-1,4-naphthoquinones, and Related Compounds

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Series of (1,4)-Naphthoquinono(3,2-c)-1H-pyrazoles and 2-substituted amino-1,4-naphthoquinones have been synthesised and studied for their possible anticancer activity (animal tumours, *Walker* 256 carcinosarcoma), Influenza RNA transcriptase activity, antibiotic activity (*C. neoformans, T. mentagraphytes, M. canis, A. niger*, and *C. albicans*).

Synthese und pharmakologische Prüfung einiger (1,4)-Naphthochinon-[3,2-c]-1H-pyrazole, 2-substituierter Amino-1,4-naphthochinone und verwandter Verbindungen

1,4-Naphthochinon[3,2-c]-1H-pyrazole und 2-substituierte Amino-1,4-naphthochinone wurden hergestellt und auf cytostatische Wirkung (Tiertumore, *Walker* 256 Sarkom)), Influenza RNS-Transkriptase Aktivität und antibiotische Wirksamkeit (*C. neoformans, T. mentagraphytes, M. canis, A. niger* und *C. albicans*) geprüft.

An o-aminoquinonoid unit is found in a number of antitumour antibiotics, e.g. streptonigrin, actinomycin, mitomycins, etc. To incorporate an *o*-aminoquinonoid unit and in view of some salient structural features of *Arnebins*[1,2], the synthesis of compounds **2, 3**, and **4** have been carried out to study their anticancer activity against *C. neoformans, T. mentagraphytes, M. canis, A. niger*, and *C. albicans*.

Chemistry

The synthetic route to the title compounds is shown in Scheme 1. Thus Naphthoquinones **1**, prepared by known procedures, on condensation with secondary amines in EtOH gave 2-substituted amino-1,4-naphthoquinones **3**, whereas with diazomethane and diazoacetic ester, it formed compounds **2**. Compound **1** (X=X'=H; R=H) on refluxing with 2-methylindole in EtOH gave compound **4**.

Experimental Part

Melting points: Townson and Mercer apparatus. - IR-spectra: Perkin-Elmer Model 137. - ^{1}H-NMR-spectra: Varian A-60 D, TMS as internal standard, δ (ppm), J in Hz. - TLC: silica gel G or basic Al_2O_3 plates. - Temp. in °C.

Table 1

Compound No.	X	X'	R	N<	Yield %	M.P. °C	Molecular formula		Analysis Found %	Calcd. %
3a	OH	OH	H	1-Pyrrolidino-	39	>300	$C_{14}H_{13}NO_4$	C	64.4	64.9
								H	5.32	5.02
								N	5.0	5.4
3b	OH	OH	H	1-Piperidino-	37	>320	$C_{15}H_{15}NO_4$	C	65.7	65.9
								H	5.45	5.49
								N	5.8	5.1
3c	OH	OH	Me	1-Ethyleneimino-	50	>320	$C_{13}H_{11}NO_4$	C	63.9	63.7
								H	4.63	4.48
								N	5.8	5.7
3d	OH	OH	Me	1-Pyrrolidino-	74	>320	$C_{15}H_{15}NO_4$	C	65.7	65.9
								H	5.50	5.49
								N	4.7	5.1
3e	OH	OH	Me	1-Piperidino-	70	>320	$C_{16}H_{17}NO_4$	C	66.1	66.5
								H	5.50	5.92
								N	4.5	5.0
3f	OH	OH	Me	N-4-Methyl-N-Piperazino-	53	>320	$C_{16}H_{18}N_2O_4$	C	63.4	63.2
								H	5.68	5.96
								N	8.9	9.3

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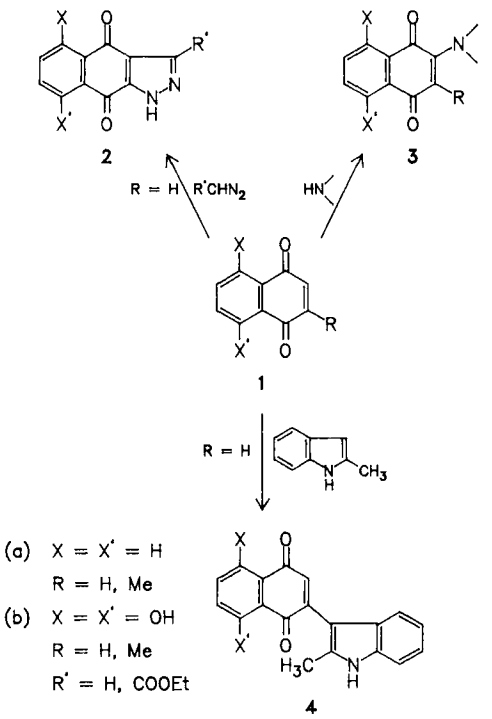

Scheme 1

5-Hydroxy-8-chloro-1,4-naphthoquinone (8-Chlorojuglone) (1, X=OH, X'=Cl, R=H)

A mixture of p-chlorophenol (8.40 g; 0.66 mole) and maleic anhydride (16.16 g, 1.70 mole) was added to a melt of anhydrous $AlCl_3$ (160 g) and NaCl (32 g) kept at 180° with vigorous stirring. After the addition, the temp. was raised to 200° for 5 min, cooled to 120° and the mixture was poured onto HCl-ice. After standing for a few h, the product was filtered, washed with H_2O, dried and extracted with petroleum ether in a Soxhlet apparatus, when **1** (X=OH, X'=Cl, R=H) was obtained as petroleum ether soluble fraction, yield 3.3 g (24%), mp. 201° (lit.[3]: 201-2°). - IR (KBr) cm^{-1}: 1650 (H-bonded-C=O); 1670 (-C=O). - ^{1}H-NMR ($CDCl_3$): 7.01 (s, H-2, H-3), 7.28 (d, J = 8.5 Hz, H-6), 7.71 (d, J = 8.5 Hz, H-7).

5,8-Dihydroxy-1,4-naphthoquinone (naphthazarin) (1, X=X'=OH, R=H)

It was prepared in a similar manner, yield 27%, mp. 275-78° (lit.[4]: 276-80°). - IR (KBr) cm^{-1}: 1610 (H-bond-C=O). - ^{1}H-NMR ($CDCl_3$): 7.18 (s, H-2, H-3, H-6, H-7), 12.4 (broad, 5-OH, 8-OH, exchangeable with D_2O).

2-Methyl-5,8-dihydroxy-1,4-naphthoquinone (1, X=X'=OH,R=Me)

It was prepared by a similar procedure, yield 30%, mp. 175-6° (lit.[5]: mp. 173°).

(1,4)-Naphthoquinono[3,2-c]-1H-pyrazole (2, X=X'=R'=H)

Diazomethane in ether (30 ml) was added to a solution of 1,4-naphthoquinone (1.58 g, 0.01 mole) in ether (100 ml) under ice cooling when a yellow crystalline product separated after a few min, which was filtered and crystallized from acetic acid; yield 1.78 g (89%), mp. 356° (lit.[6]: mp. 349°).

3-Carbethoxy-5,8-dihydroxy-(1,4)-naphthoquinono[3,2-c]-1H-pyrazole (2, X=X'=OH, R'=COOEt)

Ethyl diazoacetate (1.14 g, 0.01 mole) was added to a solution of naphthazarin (1.90 g, 0.01 mole) in ether (200 ml) and allowed to stand between 0-4° for 48 h; then the mixture was concentrated to about 20 ml. The product which separated was crystallized from EtOH to give a light brown solid, yield 1.5 g (50%), mp. 239-40°. - IR (KBr) cm^{-1}: 1636 (C=O); 1723 (-CO-OEt); 3248 (-OH). - $C_{14}H_{10}N_2O_6$ (302.2) Calcd. C 56.3 H 3.31 N 9.3 Found C 56.0 H 3.80 N 9.2.

1-Pyrrolidino-5,8-dihydroxy-1,4-naphthoquinone (3, X=X'=OH; R=N-pyrrolidino)

Pyrrolidine (0.71 g, 0.01 mole) was added to naphthazarin (0.47 g, 0.0025 mole) in absol. EtOH (40 ml). The mixture was kept for 72 h at room temp. The solid product which separated was crystallized from absol. EtOH, yield = 39%, mp. >300°. - IR (KBr) cm^{-1}: 1640 (C=O); 3400 (-OH). - $C_{14}H_{13}NO_4$ (259.2) Calcd. C 64.9 H 5.02 N 5.4 Found C 64.4 H 5.32 N 4.9.

Similarly, compounds **3a-f** were prepared, their characterization data are recorded in Table 1.

2-(2-Methyl-3-indolyl)-1,4-naphthoquinone (**4**)

A solution of 1,4-naphthoquinone (1.58 g, 0.01 mole) and 2-methylindole (1.31 g, 0.01 mole) in EtOH (50 ml) was refluxed for 6 h and left for 3 days at room temp. The dark violet coloured solid which separated was crystallized from EtOH, yield 0.96 g (30%), mp. 174-76° (lit.[7]: mp. 176-8°). - IR (KBr) cm^{-1}: 3323 (-NH); 1657 (C=O). - $C_{19}H_{13}NO_2$ (287.3) Calcd C 79.4 H 4.52 N 4.9 Found C 79.3 H 4.68 N 4.4.

Pharmacology

Anticancer-Screening

The compounds were treated against animal tumours (*Walker* 256 Carcinosarcoma) following the standard procedure[2a]. The tumour was implanted in the right thigh muscle of the rat with *Walker* 256 Carcinosarcoma cells. The treatment was given from 3rd to 6th day of tumour implantation intraperitoneally. On the 7th day, both hind legs of the rats were removed from the hip joint and weighed. By substracting the weight of the normal left leg from the tumour bearing right leg, the weight of the net tumour was obtained.

Screening against nucleic acid polymerising enzymes - Reverse transcriptase Inhibition (RNA-dependent DNA polymerase)-

In this test, virion contained RNA dependent DNA polymerase from Rauscher murine leukemia virus was activated and assayed by the method of *Baltimore* and *Smoller*[8].

Influenza RNA transcriptase

The inhibition against influenza enzyme was measured by following the method of Compans and Caliguiri[9].

Antibiotic Screening:

The compounds were tested for their antibiotic activity by standard methods[10].

Results and Discussions

Anticancer Activity

The tumour weight of the treated group was devided by the tumour weight of the control group, the ratio is expressed as T/C. For synthetic compounds a T/C % index of 42 or less is considered indicative of drug activity. The results are summarised in Table 2.

Table 2

Compound	Dose mg/kg	Survivors	T/C %	Remarks
Naphthazarin	10	3/4	37	active
2-Methylnaphthazarin	2.5	4/4	23	active
5-Amino-1,4-naphthoquinone	2.5	4/4	112	inactive
2-Methyl-3-(1-piperidino)-naphthazarin	50	3/3	107	inactive
(1,4)-Naphthoquinono-(3,2-C)-1H-Pyrazole	50	3/3	115	inactive
2-(2-Methyl-3-indolyl)-1,4-naphthoquinone	50	3/3	87	inactive

Table 3

Compound	DPM	% Control
Control	340 ± 25	25
8-Chloro-5-hydroxy-1, 4-naphthoquinone	373 ± 15	55

1. Naphthazarin and 2-methyl naphthazarin were found to be active against *Walker* 256 carcinosarcoma.

2. In tissue culture (KB system), 5-hydroxy-8-chloro-1,4-naphthoquinone showed anticancer activity though at a high dosage, -0.95 ED_{50} 2.1 x 10.

Activity against nucleic acid polymerising enzymes

Some compounds have been tested involving nucleic acid polymerising enzymes: WSN influenza, poliovirus induced RNA-dependent RNA polymerase, infleunza RNA transcriptase, and RNA-dependent DNA polymerase.

Reverse Transcriptase inhibition (RNA-dependent DNA Polymerase)

8-Chloro juglone showed 97% activity at concentration of 56 μM. Other compounds did not show any significant activity.

RNA- Dependent RNA polymerase activity

This enzyme is induced in poliovirus type 2 infected Hela cells. The activity peaks 6 h after injection and is found associated with the membranes. The cells are disrupted at this stage by gentle homogenisation after hypotonic swelling and the membranes are differentially centrifuged from debris and nuclei. The assay measures ^{3}H-uridine triphosphate incorporation. The results are summarised in Table 3. None of the other compounds tested showed any inhibition against WSN influenza virus on MDBK cells.

Influenza RNA transcriptase Activity

No inhibition by these compounds was found against intact influenza or polio system. The results against influenza enzyme are given in Table 4.

Table 4

Compound	% Inhibition at 10 μg/ml
Naphthazarin	129
2-(1-Ethyleneimino)-3-methyl-5,8-dihydroxy-1,4-naphthoquinone	120
2-Pyrrolidino-3-methyl-5,8-dihydroxy-1,4-naphthoquinone	107

Table 5

Compound	*C. neoformans*	*T. mentagraphytes*	*M. canis*	*A. niger*	*C. albicans*
2-(2-methyl)-3-indolyl-1,4-naphthoquinone	12.5	25.0	12.5	25.0	-

Antibiotic Activity

The compounds were tested for their antibiotic activity by standard methods[10] and those showing activity are listed in Table 5.

Thus, these compounds do show weak to strong inhibition of different biological systems.

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