Antitumor Agents. 89.[1] Psychorubrin, a New Cytotoxic Naphthoquinone from *Psychotria rubra* and Its Structure–Activity Relationships

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A new naphthoquinone, isolated from the alcoholic extract of *Psychotria rubra*, exhibited significant cytotoxicity in the KB cell assay (ED_{50} = 3.0 μg/mL). Spectral data was used to assign the structure of psychorubrin as **2**. Naphthoquinone derivatives **6**, **8**, **13**, and **14** were prepared and exhibited superior cytotoxic activity to that of psychorubrin. All were potential Michael acceptors whose conjugation had been extended. However, when a hydrophilic hydroxy group was present in such compounds, reduced in vitro activity was observed.

In the preliminary screening of crude extracts of Formosan plants, the alcoholic extracts of *Psychotria rubra*, which is known as "Chiu Chieh Mu" in Chinese folk medicine, showed significant reproducible inhibitory activity against KB cells. As a result of separation of the alcoholic extract, guided by an in vitro assay in KB cells, a new naphthoquinone named psychorubrin and known helenalin were isolated as the active principles. The present paper describes the isolation and structure elucidation of this new naphthoquinone and the structure–activity relationships of a series of related synthetic naphthoquinone derivatives.

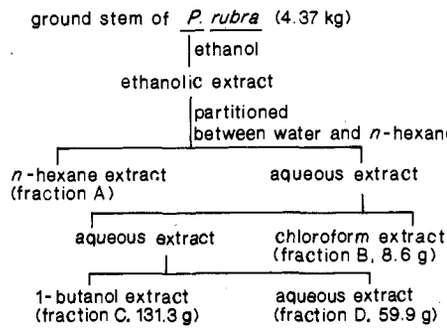
Isolation and Structure of Psychorubrin

The ground stem of *P. rubra* was extracted as shown as Scheme I to give fractions A–D. A KB cell assay of each fraction showed significant cytotoxic activity in the chloroform fraction B (ED_{50} = 11 μg/mL) while the other fractions were inactive (ED_{50} < 20 μg/mL). Further purification of fraction B by chromatography yielded the principal active constituents helenalin (**1**) and psychorubrin (**2**) at 0.0007% and 0.0003%, respectively. The identity of **1** was confirmed by direct comparison with an authentic sample.[2]

Compound **2**, mp 150–152 °C, $C_{13}H_{10}O_4$, $[\alpha]_D$ = 0°, was identified on the basis of its spectral properties. The ^1H NMR (Table I) (two two-proton multiplets centered at δ 8.09 (Ar H) and δ 7.73 (Ar H) and the IR spectra [1590 (C=C), 1640, 1660 (quinone C=O) cm^{-1}] indicate the presence of a naphthoquinone moiety. The UV spectrum of **2** was also characteristic of a naphthoquinone [245 (log ε 4.78), 250 (4.77), 262 (4.70), and 330 (3.99) nm].[3] The IR spectra also indicated the presence of a secondary hydroxyl group (3410 cm^{-1}). The NMR spectrum of **2** also showed the presence of a hemiacetal proton (5.50, m, H-3), an oxymethylene group (δ 4.69 dt, J = 3.5, 18.6 Hz, 1 H, H-1; 4.82, dt, J = 3.0, 18.6 Hz, 1 H, H-1) and methylene group (δ 2.73, 2.85, each a dm, J = 19.3 Hz, H-4).

The COSY spectrum of **2** showed the methylene protons δ 2.73 and 2.85 were coupled with both the hemiacetal proton at δ 5.50 and the methylene protons at δ 4.69 and 4.82. These data are consistent with the structure shown for psychorubrin as in **2**. Further proof was provided by derivitization of **2** by dehydration with *p*-toluenesulfonic acid to give **4**. The NMR spectrum of **4** exhibited a pair of doublets at δ 6.99 and 6.13 (J = 6.0 Hz, H-3,4) and a two-proton multiplet at δ 5.18 (H-1). The UV spectrum of **4** suggested that the newly appeared double bond was conjugated with the double bond of the quinone (400 and 420 nm).

Scheme I. Fractionation of Cytotoxic Antitumor Alcoholic Extract of *P. rubra*



Additional structure proof was provided by the preparation of compounds **7** and **8** whose NMR spectra were compared with those of **2** and **4**, respectively. As can be seen (Table I), they were quite different from each other. The structure of psychorubrin was thus proven indirectly.

When compound **8** was tested in the KB cell assay, it was shown to exhibit greater activity than psychorubrin and as a result a number of analogues of **8** were prepared in order to elucidate the structure–activity relationships.

Chemistry

For the purpose of structure elucidation, psychorubrin was acetylated under standard conditions to give **3** and dehydrated in the presence of *p*-toluenesulfonic acid to give **4**. The synthetic analogues all arose from commercially available 2-hydroxy-1,4-naphthoquinone (**5**). Condensation of **5** with acrolein under acidic conditions gave predominately the dehydrated aldol product **6** (20%) as a mixture of cis and trans isomers and lesser amounts of the tricyclic **7** (7%), which could be separated by column chromatography (Scheme II). Compound **7** was dehydrated with *p*-toluenesulfonic acid to give **8**, which arose via isomerization of the initially formed double bond. Transformation of the 2-hydroxyl group of **7** yielded **9**–**11** by standard methods.

2-Hydroxy-1,4-naphthoquinone (**5**) was also condensed with propionaldehyde to give the previously 2-hydroxy-3-(2-propenyl)-1,4-naphthoquinone (**12**) as a mixture of the cis and trans isomers.[4] Cyclization of **12** was effected with DDQ to give **8** and the previously prepared **13** and **14**. These could be separated by column chromatography.[5–8] Catalytic reduction of **8** over Pt/C gave **15** in good yield.

(1) For paper 88, see: Lee, K. H.; Lin, Y. M.; Wu, T. S.; Zhang, D.; Hayashi, T.; Chang, J.; Wu, R. Y.; Yang, T. H. *Planta Medica*, submitted.
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Table I. ^1H NMR Data of Naphthoquinones

compd	position 1	2	3	4	6,7,8,9
2	4.69 (dt, J = 3.5, 18.6 Hz) 4.82 (dt, J = 3.0, 18.6 Hz)	-	5.50 (m)	2.73 (dm, J = 19.3 Hz) 2.85 (dm, J = 19.3 Hz)	7.74 (m) 8.08 (m)
4	5.18 (m)	-	6.99 (d, J = 6.0 Hz)	6.13 (d, J = 6.0 Hz)	7.75 (m), 8.09 (m)
7		5.55 (m)	1.95 (m), 2.19 (m)	2.74 (m)	7.09 (m), 8.08 (m)
8		6.78 (ddd, J = 1.8, 2.7, 9.0 Hz)	5.15 (m)	5.88 (m)	7.78 (m), 8.13 (m)

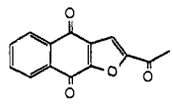
Table II. Cytotoxicity in the KB Cell Culture Assay of Psychorubrin and Related Naphthoquinone Derivatives

compd	ED_{50}, μg/mL	compd	ED_{50}, μg/mL
2	3.0	**12**	14.0
3	5.0	**13**	0.3
6	0.4	**14**	0.6
7	20.0	**15**	8.0
8	0.4	**16**	5.1
9	10.0	**17**	6.5
10	9.3	**18**	5.1
11	11.0		

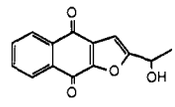
Acylation of **12** under standard conditions gave the esters **16–18**.

Results and Discussion

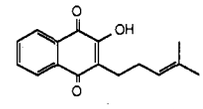
The activity of the synthetic and natural naphthoquinones discussed above in the KB cell culture assay is shown in Table II. It is not surprising that many of the compounds tested were indeed cytotoxic. Previous reports attest to the effectiveness of related naphthoquinones against KB cells. Structurally related compounds **19** (ED_{50} = 1.0 μg/mL) and **20** (ED_{50} = 2.0 μg/mL) isolated by Kingston along with lapachol (**21**) (ED_{50} = 4.4 μg/mL) and many of its derivatives have all shown significant in vitro activity.[9,10]



19



20



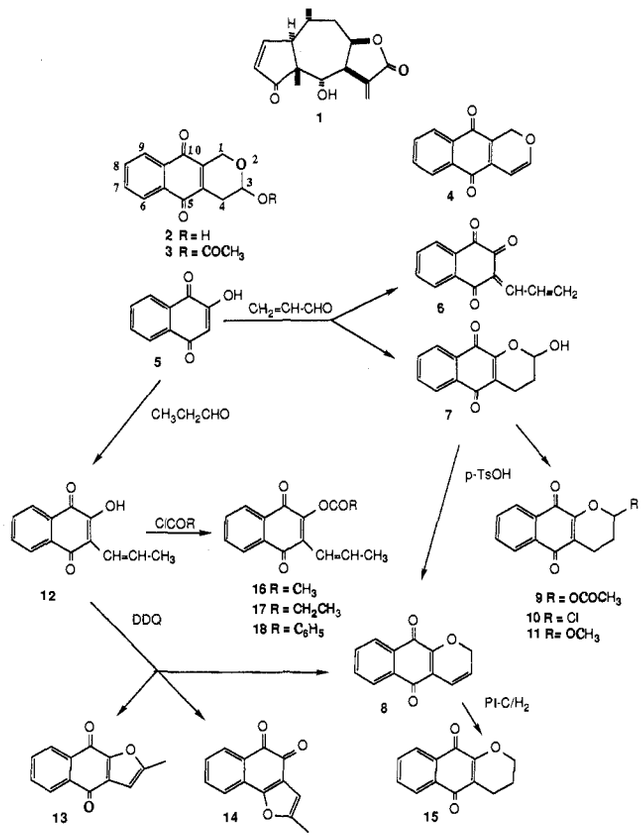
21 (lapachol)

The conjugation originally present in 1,4-naphthoquinone has been extended in all of the most active compounds (**6**, **8**, **13**, **14**). It does not appear important that the 1,4-naphthoquinone nucleus remains intact for good activity as long as extended conjugation is present (**6**, **14**). An extension of conjugation is, however, not sufficient for good in vitro activity as demonstrated by **12** which is not active. Apparently other factors are also important. In order to test the importance of masking the hydroxyl group of **12** three esters were prepared (**16–18**). The more hydrophobic esters (**16–18**) did exhibit increased activity compared with **12** but were not among the most potent compounds prepared. Variations in the acyl group present appeared to have little effect upon activity.

Experimental Section

Melting points were determined on a Fischer-Johns or a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were taken of a Perkin-Elmer Model 257 grating infrared spectrophotometer. UV spectra were recorded on a Varian 2290 spectrophotometer utilizing methanol solutions. NMR spectra were determined in $CDCl_3$ with a JNM-FX60 FT NMR spectrometer and a Bruker WM 250 NMR spectrometer (tetramethylsilane was used as the internal standard). Mass spectra were determined on a V.G. Micromass 7070 instrument at 70 eV with a direct inlet system. Specific rotations were taken on a

Scheme II



Autopol III automatic polarimeter (Rudolph Research). Silica gel was used for column chromatography (Merck Kieselgel 60, 230–400 mesh), TLC (Merck, silica gel 60F-254), and preparative TLC (silica gel GF, 500 and 1000 μm). Elemental analysis were performed by Analtech, Inc. Tucson, AZ.

Biological Screens. The cytotoxicity screening of the naphthoquinones was conducted according to the NIH protocol.[11] Drugs were tested from 0.1 to 20 μg/mL (N = 4) and were incubated with 10^4 tumor cells grown in MEM + 10% fetal calf serum with penicillin and streptomycin administered on day 3. The number of viable cells as determined by the trypan blue exclusion method and expressed as a percentage of those viable cells from the 0.05% Tween 80–water control. The ED_{50} values were estimated from a semi-log plot of the drug concentration (μg/mL) vs the percent of viable cells.

Isolation of Helenalin (1) and Psychorubrin (3-Hydroxy-1*H*-3,4-dihydronaphtho[2,3-*c*]pyran-5,10-dione, 2) from *P. rubra*. The *P. rubra* used was collected in Taiwan. The ground, air-dried stem (4.37 kg) was extracted with ethanol at room temperature. The combined ethanol extracts were evaporated and partitioned as described in the text. Guided by the bioassay in KB cells (Scheme I), the final active chloroform extract (8.6 g, fraction B) was redissolved in chloroform and the soluble fraction (3.0 g) was chromatographed on silica gel (250 g, 3.5 × 72 cm column) with chloroform as the eluting solvent. Fractions of 100 mL each were collected. Fractions 9–17 (R_f 0.40 in

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$CHCl_3$–MeOH, 9:1) were combined, and the solvent was evaporated off. The residue (566 mg) was subjected to preparative TLC ($CHCl_3$–MeOH, 95:5), which showed five bands as visualized under UV light. Band IV yielded colorless crystal I by further purification by preparative TLC and recrystallization from MeOH (30 mg). Crystal I, mp 168–169 °C, was identified as helenalin (1) by the comparison of IR and NMR spectra of an authentic sample. Band V was rechromatographed on silica gel (15 g, 2 × 11.5 cm column) after extraction with chloroform. Methylene chloride was used as the eluting solvent. The fractions that showed a single spot (R_f 0.44) were combined. Evaporation of the solvent yielded psychorubrin (2) as a pale yellow solid (12 mg): IR ($CHCl_3$) 3410 (OH), 1660 (quinone C=O), 1640 (quinone C=O), 1590 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ 2.73 (dm, J = 19.3 Hz, 1 H, H-4), 2.85 (dm, J = 19.3 Hz, 1 H, H-4), 2.88 (br s, 1 H, OH), 4.69 (dt, J = 3.5, 18.6 Hz, 1 H, H-1), 4.82 (dt, J = 3.0, 18.6 Hz, 1 H, H-1), 5.50 (m, 1 H, H-3), 7.74 (m, 2 H, Ar H), 8.09 (m, 2 H, Ar H); MS, m/z 212.0482 ($M^+ - H_2O$, $C_{13}H_8O_3$ requires 212.0473).

Psychorubrin Acetate (3-Acetoxy-1*H*-3,4-dihydronaphtho[2,3-*c*]pyran-5,10-dione, 3). Psychorubrin (2, 4 mg) was acetylated with acetic anhydride (0.5 mL) and pyridine (0.5 mL) for 15 h at room temperature. To this solution was added 10 mL of ether. The resulting solution was washed with 3 × 3 mL of 1.0 N HCl. The ether was evaporated under reduced pressure to give a residue, which was recrystallized from methanol to yield 3 mg (63%) of 3: mp 145–150 °C; IR ($CHCl_3$) 1745 (ester C=O), 1665 (quinone C=O), 1595 cm^{-1} (C=C); UV (log ϵ) 254 (4.81), 273 (4.32), 324 nm (3.82); $[\alpha]^{23}_D$ +0 (c 0.009, MeOH); ^1H NMR ($CDCl_3$) δ 2.86 (m, 2H, H-4), 4.66 (dt, J = 2.3, 18.8 Hz, 1 H, H-1), 4.82 (dt, J = 3.0, 18.8 Hz, 1 H, H-1), 6.43 (dd, J = 2.6, 3.7 Hz, H-3), 7.75 (m, 2 H, Ar H), 8.11 (m, 2 H, Ar H); MS, m/z 212.0472 ($M^+ - 60$, $C_{13}H_8O_3$ requires 212.0472).

1*H*-Naphtho[2,3-*c*]pyran-5,10-dione (4). A solution of psychorubrin (2, 2 mg) in dry benzene (1.5 mL) was refluxed with p-toluenesulfonic acid (4 mg) for 20 min. After cooling, the reaction mixture was filtered. The filtrate was concentrated and the residue was purified by column chromatography on silica gel (3 g, 1.1 × .65 cm column) with benzene as the eluting solvent to yield 1.5 mg (81%) of 4 as orange crystals: IR ($CHCl_3$) 1670 (quinone C=O), 1648 (quinone C=O), 1598 cm^{-1} (C=C); UV (log ϵ) 231 (3.78), 246 (3.80), 251 (3.80), 260 (3.75), 400 (2.67), 420 nm (2.37); ^1H NMR ($CDCl_3$) δ 5.18 (m, 2 H, H-1), 6.13 (d, J = 6.0 Hz), 6.99 (d, J = 6.0 Hz, 1 H, H-3), 7.75 (m, 2 H, Ar H), 8.09 (m, 2 H, Ar H); MS, m/z 212.0465 (M^+, $C_{13}H_8O_3$ requires 212.0473).

Preparation of Demethyl-α-lapachone Derivatives 6 and 2-Hydroxy-1*H*-3,4-dihydronaphtho[2,3-*b*]pyran-5,10-dione (7). A solution of 2-hydroxy-1,4-naphthoquinone (5, 2 g) in glacial acetic acid (35 mL) was heated to 80 °C and concentrated hydrochloric acid was added, followed at once by the addition of acrolein (5 mL). The reaction mixture was stirred at 75–80 °C for 1.5 h and poured into water (200 mL). The solution was extracted with benzene. After evaporation of solvent, the residue was column chromatographed on silica gel (100 g) with benzene as the eluting solvent. The eluate from benzene was evaporated to dryness. Recrystallization of the residue from methanol afforded orange prisims of 6 (495 mg, 20%) as a mixture of cis and trans isomers: mp 140–145 °C; IR ($CHCl_3$) 1670 (quinone C=O), 1645 (quinone C=O), 1635 (quinone C=O), 1588 (C=C), 1570 cm^{-1} (C=C); UV (log ϵ) 245 (4.13), 250 (4.14), 274 (4.07), 332 (3.31), 440 nm (2.96); ^1H NMR ($CDCl_3$) δ 5.12 (m, 2 H, $=CH_2$), 5.88 (dt, J = 3.6, 10.2 Hz, 1 H, $CH=CH_2$), 6.77 (dt, J = 1.5, 10.2 Hz, 1 H, $CH=CHCH=CH_2$), 7.75 (m, 2 H, Ar H), 8.10 (m, 2 H, Ar H); MS, m/z 212.0466 (M^+, $C_{13}H_8O_3$ requires 212.0473). After elution of 6, benzene–ethyl acetate (9:1) was used as the eluting solvent. Evaporation of eluate gave yellow crystals. Recrystallization from ethanol yielded yellow prisims of 7 (184 mg, 6.9%): mp 146-147 °C; IR (KBr) 3430 (OH), 1665 (quinone C=O), 1640 (quinone C=O), 1595 cm^{-1} (C=C); UV (log ϵ) 247 (sh, 4.35), 251 (4.39), 278 (sh, 4.21), 281 (4.21), 332 (3.49), 382 nm (sh, 3.11); ^1H NMR ($CDCl_3$) δ 1.95–2.19 (m, 2 H, H-3), 2.74 (m, 2 H, H-4), 5.55 (m, 1 H, H-2), 7.78 (m, 2 H, Ar H), 8.08 (m, 2 H, Ar H); MS, m/z 230.0578 (M^+, $C_{13}H_{10}O_4$ requires 230.0575). Anal. ($C_{13}H_{10}O_4$).

2-Acetoxy-2*H*-3,4-dihydronaphtho[2,3-*b*]pyran-5,10-dione (9). Compound 7 (5 mg) was acetylated with acetic anhydride (0.1 mL) and pyridine (0.1 mL) for 15 h at room temperature. To this solution was added 10 mL of ether and the resulting solution was washed with 3 × 3 mL of 1.0 N HCl. The ether layer was dried and evaporated to give a residue, which was recrystallized from methanol to yield 3.5 mg (61%) of 9 as pale yellow needles: mp 145–146 °C; IR ($CHCl_3$) 1750 (ester C=O), 1655 (quinone C=O), 1630 (quinone C=O), 1595 cm^{-1} (C=C); UV (log ϵ) 244 (4.65), 250 (4.68), 274 (sh, 4.49), 279 (4.51), 332 mm (3.81); ^1H NMR ($CDCl_3$) δ 2.03 (m, 1 H, H-3), 2.10 (s, 3 H, $COCH_3$), 2.20 (m, 1 H, H-3), 2.60 (ddd, J = 6.8, 11.3, 19.5 Hz, 1 H, H-4), 2.84 (ddd, J = 3.4, 6.4, 19.5 Hz, 1 H, H-4), 6.69 (t, J = 2.6 Hz, H-2), 7.78 (m, 2 H, Ar H), 8.11 (m, 2 H, Ar H). Anal. ($C_{15}H_{12}O_5$) C, H.

2*H*-Naphtho[2,3-*b*]-pyran-5,10-dione (8), 2-Methylnaphtho[2,3-*b*]furan-4,9-dione (13), and 2-Methylnaphtho-[1,2-*b*]furan-4,5-dione (14). A mixture of 2-hydroxy-3-(2-propenyl)-1,4-naphthoquinone (12) (150 mg), DDQ (200 mg), and benzene (20 mL) was refluxed for 2 h. After cooling, the reaction mixture was filtered. The filtrate was concentrated and the residue was column chromatographed on silica gel (30 g), eluting with benzene. The first eluate from benzene was evaporated to dryness and recrystallized from methanol to afford the previously prepared 13 (56 mg, 37.7%) as yellow needles: mp 245–246 °C (lit. mp 254–247 °C); IR ($CHCl_3$) 1670 (C=O), 1595 cm^{-1} (C=C); UV (log ϵ) 250 (4.71), 293 (3.99), 335 (3.66), 383 nm (3.53); ^1H NMR ($CDCl_3$) δ 2.56 (s, 3 H, CH_3), 6.64 (s, 1 H, H-3), 7.78 (m, 2 H, Ar H), 8.18 (m, 2 H, Ar H); MS, m/z 212.0461 (M^+ $C_{13}H_8O_3$ requires 212.0473). Anal. ($C_{13}H_8O_3$) C, H.

The second eluate from benzene was evaporated to dryness and recrystallized from methanol to give 8 (16 mg, 11%) as orange prisms: mp 160–163 °C; IR ($CHCl_3$) 1670 (quinone C=O), 1650 (quinone C=O), 1595 (C=C), 1575 cm^{-1} (C=C), UV (log ϵ) 245 (4.16), 251 (4.17), 271 (4.11), 332 (3.33), 442 (3.06) nm; ^1H NMR ($CDCl_3$) δ 5.15 (m, 1 H, H-3), 5.88 (m, 1 H, H-4), 6.78 (ddd, J = 1.8, 2.7, 9.0 Hz, 2 H, H-2), 7.78 (m, 2 H, Ar H), 8.13 (m, 2 H, Ar H); MS, m/z 212.0464 (M^+ $C_{13}H_8O_3$ requires 212.0473).

The third eluate from benzene was evaporated to dryness and recrystallized from methanol to give the previously prepared 14 (9 mg, 6%) as dark red needles: mp 163–164 °C (lit. mp 164–165 °C); IR ($CHCl_3$) 1675 (C=O), 1595 (C=C), 1585 cm^{-1} (C=C); UV (log ϵ) 247 (4.37), 267 (4.44), 326 (3.22), 458 nm (3.21); ^1H NMR ($CDCl_3$) δ 2.43 (s, 3 H, CH_3), 6.44 (s, 1 H, H-3), 7.50 (m, 3 H, Ar H), 8.00 (m, 1 H, Ar H).

2*H*-3,4-Dihydronaphtho[2,3-*b*]pyran-5,10-dione (15). A mixture of 8 (82 mg)) and Pt/C (20 mg) was stirred in ethyl acetate (10 mL) for 30 min under hydrogen atmosphere. The solution was filtered and the solvent removed under reduced pressure. The product was taken up in dry ether (15 mL) and stirred with AgO_2 (200 mg) and anhydrous sodium sulfate (120 mg) for 1 h. The insoluble material was removed by filtration and the filtrate was concentrated. The residue was purified by column chromatography on silica gel (5 g) with chloroform as the eluting solvent. Recrystallization of the product from methanol yielded yellow needles of 15 (64 mg, 77%): mp 219–220 °C; IR ($CHCl_3$) 1675 (quinone C=O), 1645 (quinone C=O), 1590 (C=C), 1580 cm^{-1} (C=C); UV (log ϵ) 246 (4.35), 251 (4.38), 281 (4.17), 331 nm (3.47); ^1H NMR ($CDCl_3$) δ 2.02 (m, 2 H, H-3), 2.63 (t, J = 6.0 Hz, 2 H, H- 4), 4.36 (T, J = 5.3 Hz, 2 H, H-2), 7.70 (m, 2 H, Ar H), 8.10 (m, 2 H, Ar H); MS, m/z 214.0630 (M^+, $C_{13}H_{10}O_3$ requires 214.0623). Anal. ($C_{13}H_{10}O_3$) C, H.

2-Chloro-2*H*-3,4-dihydronaphtho[2,3-*b*]pyran-5,10-dione (10). Thionyl chloride (0.3 mL) was added to a solution of 7 (30 mg) and stirred overnight. The reaction mixture was poured into ice–water and extracted with chloroform. The solvent was removed under reduced pressure and the residue was purified by preparative TLC to afford pale yellow crystals of 10 (9 mg, 28%): mp 165–167 °C; IR ($CHCl_3$) 1655 (quinone C=O), 1630 (quinone C=O), 1595 (C=C), 1585 cm^{-1} (C=C); UV (log ϵ) 244 (4.32), 249 (4.36), 279 (4.19), 333 nm (3.41); ^1H NMR ($CDCl_3$) δ 2.35 (m, 2 H, H-3), 2.82 (m, 2 H, H-4), 6.54 (m, 1 H, H-2), 7.73 (m, 2 H), 8.11 (m, 2 H); MS, m/z 248.0242 (M^+, $C_{13}H_9O_3Cl$ requires 248.0239).

2-Methoxy-2*H*-3,4-dihydronaphtho[2,3-*b*]pyran-5,10-dione (11). Preparation of 11 was given by treatment of 7 with CH_2N_2 in the presence of boron trifluoride etherate.[12] Compound 11:

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mp 118–119 °C; IR ($CHCl_3$) 1655 (C=O), 1595, cm^{-1} (C=C); UV (log ϵ) 251 (4.19), 277 (4.04), 281 (4.04), 332 (3.30), 382 nm (3.01); ^1H NMR ($CDCl_3$) δ 1.85 (m, 2 H, H-3), 2.68 (dd, J = 6.0, 9.0 Hz, 2 H, H-4), 3.36 (s, 3 H, OCH_3), 4.49 (t, J = 6.0 Hz, 1 H, H-2), 7.65 (m, 2 H, Ar H), 8.10 (m, 2 H, Ar H); MS, m/z 244.0737 (M^+, $C_{14}H_{12}O_4$ requires 244.0735).

2-Acetoxy-3-(2-propenyl)-1,4-naphthoquinone (16). In 1.0 mL of pyridine was placed 43 μL of acetic anhydride and to this was added 100 mg of **12**. The resulting dark red solution was stirred overnight at room temperature. In the morning 25 mL of ether was added and the resulting solution was washed with 3 × 15 mL of 1.0 N HCl. The ether was dried ($MgSO_4$) and evaporated to give a residue, which was purified by column chromatography ($CHCl_3$) to give 87 mg (73%) of **16** as yellow crystals as a mixture of cis and trans isomers: mp 92–94 °C; IR (CCl_4) 1770 (ester C=O), 1660 (quinone C=O), 1625 (quinone C=O), 1590 (C=C), 1170 cm^{-1} (c—O); ^1H NMR ($CDCl_3$) δ 1.86 (d, J = 6 Hz, 3 H, C=CCH_3), 2.32 (s, 3 H, COCH_3), 6.39 (m, 2 H, CH=CHCH$_3$), 6.80 (dm, J = 6 Hz, 1 H, C=CHCH$_3$), 7.64 (m, 2 H, Ar H), 8.00 (m, 2 H, Ar H). Anal. ($C_{15}H_{12}O_4$) C, H.

2-(Propionyloxy)-3-(2-propenyl)-1,4-naphthoquinone (17). To 1.0 mL of pyridine was added 48 μL of propionyl chloride and to this was added 100 mg of **12**. The resulting dark red solution was allowed to stir for 1 h at room temperature. Initial attempts to remove the excess pyridine via an aqueous wash led to decomposition of the product. Therefore, the pyridine was removed under reduced pressure and the product was purified by column chromatography to give 10 mg (7.8%) of **17** as yellow crystals as a mixture of cis and trans isomers: mp 59–61 °C; IR (CCl_4) 1770 (ester C=O), 1670 (quinone C=O), 1630 (quinone C=O), 1595 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ 1.32 (t, J = 7 Hz, 3 H, CH_2CH_3), 1.98 (d, J = 6 Hz, 3 H, = C=CCH_3), 2.74 (q, J = 7 Hz, 2 H, COCH_2), 6.54 (brs, 1 H, CH=CHCH$_3$), 6.98 (dm, J = 7 Hz, 1 H, C=CHCH$_3$) 7.70 (m, 2 H, Ar H), 8.10 (m, 2 H, Ar H). Anal. ($C_{16}H_{14}O_4$) C, H.

2-(Benzyloxy)-3-(2-propenyl)-1,4-naphthoquinone (18) was similarly prepared with benzoyl chloride (63 μL) in 20% yield and isolated as yellow crystals as a mixture of cis and trans isomers: mp 118–120 °C; IR (CCl_4) 1740 (ester C=O), 1670 (quinone C=O), 1630 (quinone C=O), 1590 (C=C), 1110 cm^{-1} (C—O); ^1H NMR ($CDCl_3$) δ 1.95 (d, J = 6 Hz, 3 H, CH_3), 6.36 (m, 1 H, CH=CHCH$_3$), 6.65 (m, 1 H, C=CHCH$_3$), 7.63 (m, 5 H, Ar H), 8.23 (m, 4 H, Ar H). Anal. ($C_{20}H_{14}O_4$) C, H.

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Carbamate Ester Derivatives as Potential Prodrugs of the Presynaptic Dopamine Autoreceptor Agonist (–)-3-(3-Hydroxyphenyl)-*N*-propylpiperidine

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Twenty derivatives bearing substituents on the phenolic function of (–)-3-(3-hydroxyphenyl)-*N*-propylpiperidine [(–)-3-PPP] were synthesized and tested as prodrugs. The carbamate ester derivatives were found to be the most suitable prodrugs, and especially the 4-isopropylphenylcarbamate **20** was capable of escaping the first-pass metabolism and still generating high plasma levels of the parent compound. Four hours after an oral dose of 100 μmol/kg to rats, a plasma level of 2400 nmol/L of (–)-3-PPP was detected by an HPLC method. This was 90 times the level reached after 4 h (27 nmol/L) when (–)-3-PPP itself was given orally at the same dose.

In our preclinical studies of the presynaptic dopamine autoreceptor agonist (–)-3-(hydroxyphenyl)-*N*-propylpiperidine [(–)-3-PPP], developed as an alternative antipsychotic agent in humans,[1] the intravenous administration of [^3H]-(–)-3-PPP to mice resulted in high concentrations in the central nervous system (CNS) shortly after administration.[2] This indicated good penetration through the blood–brain barrier. We also found that (–)-3-PPP had a low oral bioavailability in all species investigated. Following oral administration in rats, the major portion of the dose (89%) was excreted in the urine as the 3-PPP-glucuronide, which indicated that the compound was well absorbed. The reduced systemic availability following oral administration was thus due to first-pass metabolism in the intestinal mucosa and/or in the liver.

In this paper we describe the synthesis and the evaluation of a number of prodrug derivatives of (–)-3-PPP. Our aim was to design derivatives with good absorption, capable of escaping the first-pass metabolism in the hepatoportal system, and generting high plasma and tissue levels of the parent compound, (–)-3-PPP. For this purpose, a screening system was designed where rats were given 100 μmol/kg orally of the prodrug, whereupon the plasma levels of the parent compound were determined after 1 and 4 h.

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