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OXIDATION WITH FREMY'S SALT—VIII[1]

PERI-EFFECT OF A GROUP LOCATED AT THE C_5 POSITION OF 1-NAPHTHOL AND RELATED COMPOUNDS

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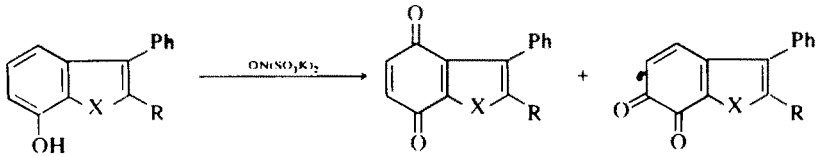
Abstract—It has been shown by a series of experiments on 5-alkyl, 5-halo, and other 5-substituted 1-naphthol derivatives that the product ratio of the *ortho*- and *para*-naphthoquinones formed on oxidation with Fremy's salt is controlled by the bulkiness of the C_5-substituent.

It is well known that Fremy's salt,[2] dipotassium nitrosodisulfonate [$ON(SO_3K)_2$], is a selective agent for oxidation of phenol and aniline derivatives to the corresponding quinones under very mild conditions, and following the numerous experimental results achieved by Teuber *et al.*,[2] this reagent has become widely accepted as a specific agent for the oxidation of phenols to *p*-quinones when the position *para* to the OH function is unsubstituted. In spite of this generalization, we have found that oxidation of 5-substituted 1-naphthol derivatives with this reagent gives an *o*-quinone derivative, with or without a *p*-quinone derivative, even in the case of 1-naphthol itself, and that this unusual transformation can be ascribed to a peri effect of the substituent on the C_5 position of the 1-naphthol portion in the starting molecule. This paper describes our experiments on this matter.

In the course of study on syntheses of heterocyclic quinones, we[3-5] occasionally found that 7-hydroxy-3-phenyl-benzo[b]-thiophene, -benzofuran and -indole derivative [(**1**)–(**5**)] were oxidized with Fremy's salt to give a corresponding *o*-quinone with or without *p*-quinone regardless of the species of the heterocyclic atom in the skelton (Table 1), while the 3-unsubstituted derivatives [(**6**)–(**8**)] gave only *p*-quinone (Table 2).

In 1953, Teuber *et al.*[6] proposed a mechanism involving three steps for the oxidative process with a simple phenol: (i) a first molecule of Fremy's salt abstracts an H atom to give a resonance-stabilized cyclohexadione radical which has three canonical forms (corresponding to formulae **V**, **Va** and **Vb**); (ii) next another molecule of the reagent attacks this intermediate (corresponding to **V**) at the position *para* to the phenolic group assuming this position is in fact free; (iii) loss of the elements of dipotassium imidobissulfate, $HN(SO_3K)_2$ (**10**), from the cyclohexadienone intermediate (corresponding to formula **VI**) then forms the *p*-quinone. Following Teuber's mechanistic proposal, we[4] explained our unusual formation of an *o*-quinone derivative in the oxidation of 3-phenyl heterocyclic phenol derivatives by supposing that, at the second stage of the oxidation attack of the reagent at the position *para* (C_4) to the phenolic group was sterically hindered by the phenyl group at C_3. On the other hand,

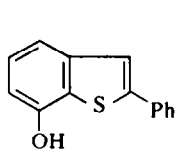
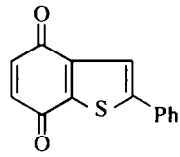
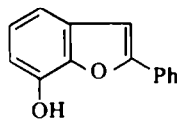
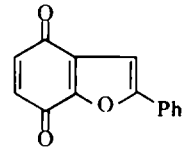
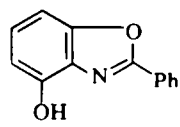
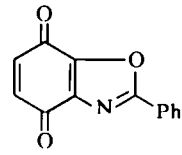
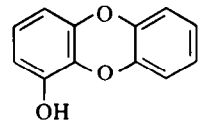
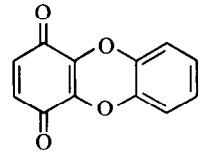
Table 1.

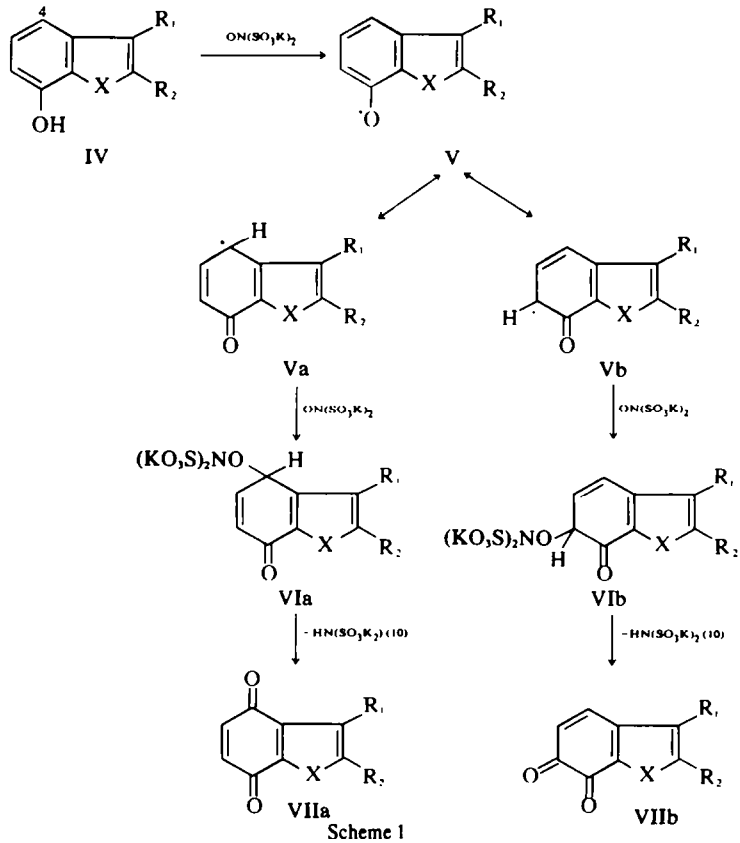


I II III

No. of starting material (I)	X	R	*p*-Quinone (II) Yield	*p*-Quinone (II) Signal pattern of quinoid Hs	*o*-Quinone (III) Yield	*o*-Quinone (III) Signal pattern of quinoid Hs	Product ratio $o/(o+p)\times 100$
1	S	H	35.3%	AB ($\Delta\nu/J = 1.5$)	40.7%	AX ($\Delta\nu/J = 11.5$)	53.6
2	S	Ph	14.2%	AB ($\Delta\nu/J = 2.0$)	45.5%	AX ($\Delta\nu/J = 9.2$)	76.2
3	O	Ph	40%	AB ($\Delta\nu/J = 0.7$)	>40%	AX ($\Delta\nu/J = 8.8$)	>50
4	NH	Ph	20%	A_2	61%	AX ($\Delta\nu/J = 12.5$)	75.3
5	NMe	Ph	0%		38.5%	AX ($\Delta\nu/J = 12.6$)	100

Table 2. Oxidation of some heterocyclic phenols with $ON(SO_3K)_2$

No.	Starting material Structure	Product Structure	Yield	Signal pattern of quinoid Hs
6			31.5%	AB ($\Delta\nu/J = 1.3$)
7			37.0%	A_2
8			*ca.* 30%	A_2
9			45.3%	A_2



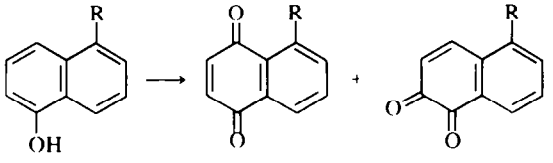
Scheme 1

about two decades ago, Teuber *et al.* reported that oxidation of 3-hydroxychrysene[7] and 1-hydroxycarbozole[8] with this reagent gave *p*-quinone derivatives in 55.1 and 76% yield, respectively. These reported results would seem to be contradictory to our conclusion, which is deduced from results on the oxidation of a 5-6 fused ring system, since theoretically a peri effect should be more clearly observed in a 6-6 fused ring system than in a 5-6 fused system. We therefore examined the oxidation of 1-naphthol itself with Fremy's salt, and that of some 5-substituted derivatives. The starting 5-alkyl-1-naphthol derivatives[9] used in this study were prepared by treatment of 5-methoxy-8-chloro-1-tetralone with the corresponding Grignard reagents or alkyl lithium followed by dehalogenation and dehydrogenation. 5-Fluoro-, 5-chloro and 5-iodo-1-naphthols[1] [(**24**), (**27**) and (**33**)] were obtained from the diazonium salt of 5-methoxy-1-naphthylamine via Siemann reaction, Sandmeyer reaction, and treatment with potassium iodide, respectively. 5-Bromo-, 5-methoxy-, 5-nitro- and 5-acetylamino-1-naphthols[1] [(**30**), (**36**), (**39**) and (**42**)] were prepared by known processes. The quinone mixtures obtained by the general procedure (Experimental) were separated into their components by column chromatography on silicic acid in the dark. Structural assignments of the isolated quinones were dependent upon the observation that the signals due to the quinoid protons appeared as an A_2 or AB type ($\Delta\nu/J = 0$–3 Hz) in *p*-quinone derivatives but as an AX type ($\Delta\nu/J = 8$–13 Hz) in *o*-quinone derivatives.[5] Table 3 summarizes our experimental results obtained on oxidation of the series of 5-alkyl-, 5-halo-1-naphthols and other related compounds with Fremy's salt.

It should be noted here that our examination of the oxidation of 1-naphthol itself (**11**) with this reagent gave *o*-naphthoquinone (**13**) in 15.1% yield with *p*-naphthoquinone (**12**) in 74.9% yield, although Teuber *et al*[10] reported in 1954 that the same reaction afforded *p*-naphthoquinone (**12**) in 91% yield as sole product. In our run, the possibility that the *o*-naphthoquinone (**13**) formed came from 2-naphthol contaminating the 1-naphthol can be excluded, because the starting 1-naphthol[9] used in the experiment was freshly prepared in our laboratory from 1-tetralone by dehydrogenation with 30% Pd-C in *p*-cymene. The result indicates that the formation of *o*-naphthoquinone derivative is common in the oxidation of an 1-naphthol derivative with Fremy's salt. The results listed in Table 3 show that the percentage of the *o*-quinone derivative in the mixture of quinone products increased with increasing bulkiness of the substituent located at the C_5 position of the starting 1-naphthol compound.

We would like to draw attention to the following two deductions. As reported in the previous paper, oxidation

Table 3.



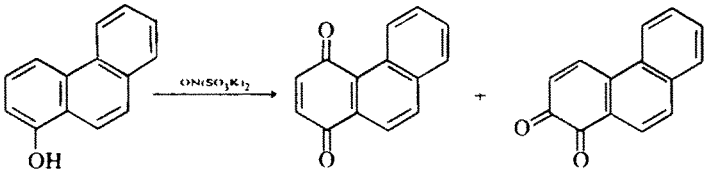
VIII IX X

No. of starting material (VIII)	R	*p*-Quinone (IX) No.	*p*-Quinone (IX) Yield	*p*-Quinone (IX) Signal pattern of quinoid Hs	*o*-Quinone (X) No.	*o*-Quinone (X) Yield	*o*-Quinone (X) Signal pattern of quinoid Hs	Product ratio $o/(o+p)\times 100$
11	H	12	74.9%	A_2	13	15.1%	AX ($\Delta\nu/J = 10.2$)	16.8
	H(Teuber's)		(91.0%)			(0%)		
14	Me	15	33.3%	A_2	16	47.0%	AX ($\Delta\nu/J = 13.5$)	58.5
17	Et	18	12.3%	A_2	19	56.0%	AX ($\Delta\nu/J = 13.2$)	82.0
20	i-Pr		0%	—	21	60.0%	AX ($\Delta\nu/J = 14.5$)	100
22	Ph		0%	—	23	65.9%	AX ($\Delta\nu/J = 12.0$)	100
24	F	25	46.3%	A_2	26	29.7%	AX ($\Delta\nu/J = 12.3$)	39.1
27	Cl	28	19.4%	A_2	29	71.1%	AX ($\Delta\nu/J = 13.0$)	78.6
30	Br	31	10.4%	A_2	32	85.6%	AX ($\Delta\nu/J = 14.6$)	89.2
33	I	34	4.2%	AB ($\Delta\nu/J = 1.2$)	35	84.6%	AX ($\Delta\nu/J = 13.1$)	95.3
36	OCH_3	37	trace	—	38	91.6%	AX ($\Delta\nu/J = 15.7$)	~100
39	NO_2	40	45.3%	A_2	41	15.0%	AX ($\Delta\nu/J = 11.8$)	24.9
42	NHAc	43	75.9%	A_2	44	18.2%	AX ($\Delta\nu/J = 13.0$)	20.4

of 1-hydroxydibenzo-*p*-dioxin[4,5] [Table 2: (9)] with Fremy's salt was confirmed to give the *p*-quinone derivative as sole product. Comparing this with the result obtained from 1-naphthol itself (**11**), we may safely deduce that even the C_5-hydrogen atom of 1-naphthol itself exhibits a peri interaction in the reaction, since 1-hydroxydibenzo-*p*-dioxin (**9**) can be assumed to be representative of a 6-6 fused ring system bearing no H atom at the position corresponding to the C_5 position of 1-naphthol. Furthermore, contrary to the result obtained with 1-naphthol itself, no evidence for the formation of an *o*-quinone derivative could be found in experiments on a 5-6 fused heterocyclic phenol system[3-5] having an H atom at the C_3 position [(**6**)–(**8**)]. These results indicate the difference between the peri effect of an H atom in a 5-6 fused ring system and one in 6-6 system. The above evidence and discussion support the correctness of our assumption concerning the formation of the *o*-quinone derivative of 1-naphthol and its related compounds with Fremy's salt.

This conclusion forced us to reexamine Teuber's results reported on the oxidation of polynuclear phenol derivatives[7,8] (*vide ante*), and we therefore examined the oxidation of 1-hydroxyphenanthrene[1] (**45**) which is the simplest model for our purpose. As expected, the oxidation gave a mixture of *p*- and *o*-quinone derivatives [(**46**) and (**47**)] in a 1:1 ratio, indicating that a fairly large peri effect should be observed in the oxidation of polynuclear phenol, if a portion of it occupies a position corresponding to the C_5 position of an 1-naphthol skeleton. Consequently, we may conclude that the bulkiness of the substituent situated at the C_5 position of the 1-naphthol portion of the substrate is the most important factor deciding the ratio of *o*- and *p*-quinone products formed in the oxidation with Fremy's salt.

Finally, since the ratios of the *o*- and *p*-quinone products were found to be smaller than expected in the oxidations of 5-nitro- (**39**) and 5-acetylamino-1-naphthol (**42**), the presence of the second factor, such as a direct interaction between a fairly polar substituent and the Fremy's salt, may be supposed. Experiments on this matter are now going on in our laboratory.



(**45**) (**46**) 46.6% A_2 (**47**) 42.4% $AX(\Delta\nu/J = 17.2)$

Scheme 2

EXPERIMENTAL

All m.ps were measured on a micro-melting hot-stage (Yanagimoto) and are uncorrected. IR, NMR, UV, and mass spectra were obtained with Hitachi EPI-G3, JNM-4H-100, Hitachi EPS-3T, and Hitachi RMU-6E spectrometers, respectively.

General procedure for oxidation with Fremy's salt. Freshly prepared Fremy's salt,[2] $ON(SO_3K)_2$, (1 g), was dissolved in a mixture of 1/6 M KH_2PO_4 buffer soln (20 ml) and H_2O (70 ml). To a soln containing 2 eqiv Fremy's salt was added a soln of 1 eqiv of starting phenol in 90–100 times its weight of MeOH. After being stirred at room temp. for *ca.* 1.5 hr and monitoring by TLC, the mixed soln was extracted with ether. Quinone was detected on TLC using Craven's reagent,[11] a *p*-quinone derivative showing up blue-violet with a larger R_f value, and an *o*-quinone derivative red-violet with a smaller R_f value. The ethereal soln was dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was dissolved in benzene and chromatographed on SiO_2 [100 mesh, Mallinckrodt Chemical Works] in the dark. The first eluant fraction gave a *p*-quinone derivative and subsequent eluant an *o*-quinone derivative.

Oxidation of 1-*naphthol* (**11**) *with Fremy's salt*

Compound 11° (500 mg), prepared by dehydrogenation of 1-tetralone with 30% Pd/C in *p*-cymene in our laboratory, was treated according to the general procedure described above.

(i) 1,4-*Naphthoquinone* (**12**). Compound **12** (410.6 mg) was isolated as yellow plates, m.p. 128–129° (lit[10] m.p. 124–125°), which were recrystallized from n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1660 (C=O). NMR(CCl_4) δ: 6.96 (2 H, s, quinoid Hs), 7.75 (2 H, m, C_6 and C_7–H), 8.07 (2 H, m, C_5 and C_8–H).

(ii) 1,2-*Naphthoquinone* (**13**). Compound **13** (83 mg) was isolated as red-orange prisms, m.p. 120–122° (dec) [lit[10] m.p. 120–121° (dec)] which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1660 (C=O). NMR($CDCl_3$) δ: 6.46 (1 H, d, J = 10.0 Hz, C_3-quinoid H), 7.48 (1 H, d, J = 10.0 Hz, C_4-quinoid H), 7.37–7.78 (3 H, m, aromatic Hs), 8.17 (1 H, dd, J_1 = 7.0 Hz, J_2 = 1.8 Hz, C_8–H). This material was identified with an authentic sample prepared from 2-naphthol by oxidation with Fremy's salt.

Oxidation of 5-*methyl*-1-*naphthol* (**14**) *with Fremy's salt*

The general procedure was carried out on 600 mg of **14**,[9] m.p. 92–94° (lit. m.p. 91–93°,[12] m.p. 98°,[13] m.p. 97°[14]).

(i) 5 - *Methyl* - 1,4 - *naphthoquinone* (**15**). Compound **15** (217.5 mg) was obtained as yellow needles, m.p. 122–123° (lit.[15] m.p. 120–121°), which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1657 (C=O). UV λ_{max}^{EtOH} mμ (log ϵ): 246.5 (4.26), 350 (3.53). NMR($CDCl_3$) δ: 2.73 (3 H, s, CH_3), 6.89 (2 H, s, quinoid Hs), 7.53–7.68 (2 H, m, aromatic Hs), 8.01 (1 H, dd, J_1 = 7.0 Hz, J_2 = 2.5 Hz, C_8–H). (Found: C, 76.91; H, 4.54. $C_{11}H_8O_2$ requires: C, 76.73; H, 4.68%).

(ii) 5-*Methyl* - 1,2 - *naphthoquinone* (**16**). Compound **16** (307.0 mg) was obtained as red needles, m.p. 160–161° (lit.[16] m.p. 155–157°), which were recrystallized from acetone–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1663 (C=O). UV λ_{max}^{EtOH} mμ (log ϵ): 257 (4.40), 372 (3.52) sh, 415 (3.54). NMR($CDCl_3$), δ: 2.48 (3 H, s, CH_3), 6.39 (1 H, d, J = 10.0 Hz, C_3-quinoid H), 7.35–7.50 (2 H, m, aromatic Hs), 7.74 (1 H, d, J = 10.0 Hz, C_4-quinoid H), 7.94 (1 H, dd, J_1 = 6.8 Hz, J_2 = 1.8 Hz, C_8–H). (Found: C, 76.90; H, 4.55. $C_{11}H_8O_2$ requires: C, 76.73; H, 4.68%).

Oxidation of 5-*ethyl*-1-*naphthol* (**17**) *with Fremy's salt*

The general procedure was carried out on 450 mg of **17**,[9] colourless prisms, m.p. 78–80°.

(i) 5-*Ethyl*-1,4-*naphthoquinone* (**18**). Compound **18** (59.8 mg) was isolated as yellow needles, m.p. 53–54°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1667 (C=O). UV λ_{max}^{EtOH} mμ (log ϵ): 249.5 (4.30), 255 (4.26), 261 (4.05), 352 (3.52). NMR(CCl_4) δ: 1.21 (3 H, t, J = 7.5 Hz, CH_3CH_2), 3.15 (2 H, q, J = 7.5 Hz, CH_3CH_2), 6.80 (2 H, s, quinoid Hs), 7.43–7.64 (2 H, m, aromatic Hs), 7.94 (1 H, dd, J_1 = 7.0 Hz, J_2 = 2.5 Hz, C_8–H). (Found: C, 77.12; H, 5.44. $C_{12}H_{10}O_2$ requires: C, 77.40; H, 5.41%).

(ii) 5-*Ethyl*-1,2-*naphthoquinone* (**19**). Compound **19**

(272.5 mg) was obtained as red needles, m.p. 58–60°, which were recrystallized from ether. IR ν_{max}^{Nujol} cm^{-1}: 1655 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 253.5 (4.35), 258 (4.36), 370 (3.43), 414 (3.47). NMR($CDCl_3$) δ: 1.27 (3 H, t, J = 7.5 Hz, CH_3CH_2), 2.84 (2 H, q, J = 7.5 Hz, CH_3CH_2), 6.42 (1 H, d, J = 10.0 Hz, C_3-quinoid H), 7.38–7.52 (2 H, m, aromatic Hs), 7.74 (1 H, d, J = 10.0 Hz, C_4-quinoid H), 7.95 (1 H, dd, J_1 = 7.0 Hz, J_2 = 2.5 Hz, C_8–H). (Found: C, 77.11; H, 5.44. $C_{12}H_{10}O_2$ requires: C, 77.40; H, 5.41%).

Oxidation of 5-isopropyl-1-naphthol (**20**) *with Fremy's salt*

5-Isopropyl-1,2-naphthoquinone (**21**). Treatment of **20**[9] (280 mg), colourless prisms, m.p. 68–69°, by the general procedure gave 180.5 mg of **21** as red-orange needles, m.p. 96–97°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1670 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 258 (4.41), 370 (3.42), 414 (3.45). NMR($CDCl_3$) δ: 1.32 (6H, d, J = 7.0 Hz, $(CH_3)_2CH$), 3.42 (1 H, quintet, J = 7.0 Hz, $(CH_3)_2CH$), 6.43 (1 H, d, J = 10.0 Hz, C_3-quinoid H), 7.38–7.67 (2 H, m, aromatic Hs), 7.88 (1 H, d, J = 10.0 Hz, C_4-quinoid H), 7.98 (1 H, dd, J_1 = 7.5 Hz, J_2 = 1.5 Hz, C_8–H). (Found: C, 77.96; H, 6.14. $C_{13}H_{12}O_2$ requires: C, 77.98; H, 6.04%).

Oxidation of 5-phenyl-1-naphthol (**22**) *with Fremy's salt*

5-Phenyl-1,2-naphthoquinone (**23**). Treatment of **22**[9] (400 mg), pale yellow prisms, m.p. 93–94°, by the general procedure gave 280.5 mg of **23** as red needles, m.p. 170–171°. IR ν_{max}^{Nujol} cm^{-1}: 1663 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 252.5 (4.41), 415 (3.35). NMR($CDCl_3$) δ: 6.32 (1 H, d, J = 10.0 Hz, C_3-quinoid H), 7.27–7.58 (7 H, m, aromatic Hs), 7.52 (1 H, d, J = 10.0 Hz, C_4-quinoid H), 8.13 (1 H, dd, J_1 = 6.5 Hz, J_2 = 2.5 Hz, C_8–H). (Found: C, 82.21; H, 4.38. $C_{16}H_{10}O_2$ requires: C, 82.04; H, 4.30%).

Oxidation of 5-fluoro-1-naphthol (**24**) *with Fremy's salt*

The general procedure was carried out on **24**[1] (400 mg), colourless needles, m.p. 135–136°.

(i) *5-Fluoro-1,4-naphthoquinone* (**25**). Compound **25** (201 mg) was obtained as yellow prisms, m.p. 159–162°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1670 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 243 (4.23), 248 (4.23), 343 (3.55). NMR($CDCl_3$) δ: 6.92 (2H, s, quinoid Hs), 7.42 (1 H, dq, $J_{6,7}$ = 7.8 Hz, $J_{6,F}$ = 11.0 Hz, $J_{6,8}$ = 1.25 Hz, C_6–H), 7.71 (1 H, sextet, $J_{6,7}$ = 7.8 Hz, $J_{7,8}$ = 7.8 Hz, $J_{7,F}$ = 5.0 Hz, C_7–H), 7.92 (1 H, dd, $J_{7,8}$ = 7.8 Hz, $J_{6,8}$ = 1.25 Hz, C_8–H). (Found: C, 68.02; H, 2.73. $C_{10}H_5O_2F$ requires: C, 68.19; H, 2.86%).

(ii) *5-Fluoro-1,2-naphthoquinone* (**26**). Compound **26** (129 mg) was obtained as red-brown needles, m.p. 161–162°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1670 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 245 (4.31), 340 (3.43), 398 (3.48). NMR($CDCl_3$) δ: 6.44 (1 H, d, J = 10.7 Hz, C_3-quinoid H), 7.23–7.60 (2 H, m, C_6 and C_7–H), 7.76 (1 H, d, J = 10.7 Hz, C_4-quinoid H), 7.90 (1 H, dd, J_1 = 7.0 Hz, J_2 = 2.0 Hz, C_8–H). (Found: C, 68.03; H, 2.71. $C_{10}H_5O_2F$ requires: C, 68.19; H, 2.86%).

Oxidation of 5-chloro-1-naphthol (**27**) *with Fremy's salt*

The general procedure was carried out on **27** (150 mg), colourless needles,[1] m.p. 132–133° (lit.[17] m.p. 135.5–136°).

(i) *5-Chloro-1,4-naphthoquinone* (**28**). The compound **28** (31.3 mg) was obtained as yellow needles, m.p. 164–165°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1667 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 237 (4.21), 246 (4.20) sh, 354 (3.41). NMR($CDCl_3$) δ: 6.97 (2 H, s, quinoid Hs), 7.62 (1 H, q, J_1 = 7.0 Hz, J_2 = 8.0 Hz, C_7–H), 7.77 (1 H, dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, C_6–H), 8.07 (1 H, dd, J_1 = 2.0 Hz, J_2 = 7.0 Hz, C_8–H). (Found: C, 62.42; H, 2.64. $C_{10}H_5O_2Cl$ requires: C, 62.36; H, 2.62%).

(ii) *5-Chloro-1,2-naphthoquinone* (**29**). The compound **29** (115 mg) was obtained as silky needles, m.p. 154–155°, which were recrystallized from benzen-n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1698, 1670 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 216 (4.40), 240 (4.54), 309 (3.48), 345 (3.38). NMR(DMSO-d_6) δ: 6.48 (1 H, d, J = 10.6 Hz, C_3-quinoid H), 7.46–7.94 (3 H, m, aromatic Hs), 7.86 (1 H, d, J = 10.6 Hz, C_4-quinoid H). (Found: C, 62.12; H, 2.62. $C_{10}H_5O_2Cl$ requires: C, 62.36; H, 2.62%).

Oxidation of 5-bromo-1-naphthol (**30**) *with Fremy's salt*

The general procedure was applied to **30**[1] (150 mg), pale yellow needles, m.p. 136–137° (lit.[18] m.p. 137°).

(i) *5-Bromo-1,4-naphthoquinone* (**31**). The compound **30** (16.5 mg) was obtained as yellow needles, m.p. 161–163°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1672 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 241 (4.30), 357 (3.47). NMR($CDCl_3$) δ: 6.94 (2 H, s, quinoid Hs), 7.52 (1 H, t, J = 7.8 Hz, C_7–H), 7.98 (1 H, dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, C_6–H), 8.11 (1 H, dd, J_1 = 7.8 Hz, J_2 = 1.5 Hz, C_8–H). (Found: C, 50.78; H, 2.16. $C_{10}H_5O_2Br$ requires: C, 50.67; H, 2.13%).

(ii) *5-Bromo-1,2-naphthoquinone* (**32**). The compound **32** (136.4 mg) was obtained as red-purple prisms, m.p. 164–166°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1667(C=O). UV λ_{max}^{EtOH} mμ (log ε): 247.5 (4.21) sh, 255 (4.24), 259.5 (4.23) sh, 402 (3.38). NMR($CDCl_3$) δ: 6.49 (1 H, d, J = 10.0 Hz, C_3-quinoid H), 7.35 (1 H, t, J = 8.0 Hz, C_7–H), 7.83 (1 H, dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, C_6–H), 7.95 (1 H, d, J = 10.0 Hz, C_4-quinoid H), 8.04 (1 H, dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, C_8–H). (Found: C, 50.61; H, 2.20. $C_{10}H_5O_2Br$ requires: C, 50.67; H, 2.13%).

Oxidation of 5-iodo-1-iodo-1-naphthol (**33**) *with Fremy's salt*

The general procedure was carried out on **33**[9] (1 g), colourless needles, m.p. 134–136° (lit.[19] m.p. 131–132°).

(i) *5-Iodo-1,4-naphthoquinone* (**34**). The compound **34** (44.3 mg) was isolated as red prisms, m.p. 173–175°, which were recrystallized from benzene-n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1667 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 213 (4.29), 246.5 (4.30), 377 (3.38). NMR($CDCl_3$) δ: 6.92 (1 H, d, J = 11.0 Hz, quinoid H), 7.05 (1 H, d, J = 11.0 Hz, quinoid H), 7.35 (1 H, t, J = 8.0 Hz, C_7–H), 8.16 (1 H, dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz, C_6–H), 8.37 (1 H, dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz, C_8–H). (Found: C, 42.66; H, 1.86. $C_{10}H_5O_2I$ requires: C, 42.28; H, 1.77%).

(ii) *5 - Iodo - 1,2-naphthoquinone* (**35**). The compound **35** (889.4 mg) was obtained as red prisms, m.p. 177–179°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1663 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 212 (4.27), 247 (4.32), 415 (3.36). NMR($CDCl_3$) δ: 6.48 (1 H, d, J = 10.2 Hz, C_3-quinoid H), 7.19 (1 H, t, J = 8.0 Hz, C_7–H), 7.82 (1 H, d, J = 10.2 Hz, C_4–quinoid H), 8.09 (1 H, dd, J_1 = 8.0 Hz, J_2 = 0.8 Hz, C_8 or C_6–H), 8.14 (1 H, dd, J_1 = 8.0 Hz, J_2 = 0.8 Hz, C_6 or C_8–H). (Found: C, 42.26; H, 1.87. $C_{10}H_5O_2I$ requires: C, 42.28; H, 1.77%).

Oxidation of 5-methoxy-1-naphthol (**36**) *with Fremy's salt*

5-Methoxy-1,2-naphthoquinone (**38**). Treatment of **36**[1] (400 mg), colourless needles, m.p. 137–139° (lit.[20] m.p. 135–136°), by the general method gave a negligible amount of **37**, less than *ca.* 1 mg, and 395.6 mg of **38**, red needles, m.p. 208–210°, which were recrystallized from acetone. IR ν_{max}^{Nujol} cm^{-1}: 1663 (C=O). UV λ_{max}^{EtOH} mμ (log ε): 255 (4.19), 376 (3.19) sh, 459 (3.57). NMR(DMSO-d_6) δ: 3.92 (3 H, s, OCH_3), 6.29 (1 H, d, J = 10.0 Hz, C_3-quinoid H), 7.40–7.58 (3 H, m, aromatic Hs), 7.86 (1 H, d, J = 10.0 Hz, C_4-quinoid H). (Found: C, 70.04; H, 4.30. $C_{11}H_8O_3$ requires: C, 70.21; H, 4.29%).

Oxidation of 5-nitro-1-naphthol (**39**) *with Fremy's salt*

The general procedure was carried out on **39**[1] (400 mg), orange needles, m.p. 168–169° (lit.[21] m.p. 165°).

(i) *5-Nitro-1,4-naphthoquinone* (**40**). The compound **40** (194.7 mg) was obtained as yellow-brown needles, m.p. 166–168°, which were recrystallized from benzene–n-hexane. IR ν_{max}^{Nujol} cm^{-1}: 1670 (C=O), 1534, 1328 (NO_2). UV λ_{max}^{EtOH} mμ (log ε): 229 (4.12) sh, 250 (4.19), 333 (3.22). NMR(DMSO-d_6) δ: 7.13 (2 H, s, quinoid Hs), 7.99–8.23 (3 H, m, aromatic Hs). (Found: C, 59.09; H, 2.49; N, 6.80. $C_{10}H_5NO_4$ requires: C, 59.12; H, 2.48; N, 6.90%).

(ii) *5-Nitro-1,2-naphthoquinone* (**41**). The compound **41** (64.4 mg) was obtained as red-brown needles, m.p. 135-137°, which were recrystallized from benzene. IR ν_{max}^{Nujol} cm^{-1}: 1707, 1675 (C=O), 1520, 1346 (NO_2). UV λ_{max}^{EtOH} mμ (log ε): 213 (4.28), 265 (3.93) sh, [400 (2.97) end absorption]. NMR(DMSO-d_6) δ: 6.54 (1 H, d, J = 10.6 Hz, C_3-quinoid H), 7.76 (1 H, dd, J_1 = 8.4 Hz, J_2 = 7.5 Hz, C_7–H), 7.79 (1 H, d, J = 10.6 Hz, C_4–quinoid H), 8.22 (1 H, dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, C_6 or C_8–H), 8.23 (1H, dd,

J_1 = 7.5 Hz, J_2 = 2.0 Hz, C_8 or C_6–H). (Found: C, 59.40; H, 2.63; N, 6.72. $C_{10}H_5NO_4$ requires: C, 59.12; H, 2.48; N, 6.90%).

Oxidition of 5-acetylamino-1-naphthol (42) with Fremy's salt

Treatment of **42**[1] (400 mg), colourless needles, m.p. 179–180° (lit.[22] m.p. 176–177°), by the general procedure gave a mixture of quinone products which was so labile that it decomposed during isolation by column chromatography. The reaction therefore was carried out using an alternative procedure. The mixture of **42** and Fremy's salt in buffer soln was kept standing at room temp. for 2 hr to give a ppt which showed a single spot corresponding to **43** on TLC. The ppt (300 mg) was collected by filtration, and the mother liquor was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was fractionated to an n-hexane soluble part and an insoluble one.

(i) *5-Acetylamino-1,4-naphthoquinone* (**43**). The ppt was combined with the n-hexane part and recrystallized from benzene to give 312 mg of **43** as orange needles, m.p. 178.5–180.5°. IR ν_{max}^{Nujol} cm^{-1}: 3210 (NH), 1700, 1670, 1650 (C=O). UV λ_{max}^{EtOH} mμ (log ϵ): 212.5 (4.48), 257 (4.32), 418 (3.56). NMR(DMSO-d_6) δ: 2.23 (3 H, s, $COCH_3$), 7.03 (2 H, s, quinoid Hs), 7.66 (1 H, dd, J_1 = 7.8 Hz, J_2 = 1.6 Hz, C_6–H), 7.79 (1 H, t, J = 7.8 Hz, C_7–H), 8.85 (1 H, dd, J_1 = 7.8 Hz, J_2 = 1.6 Hz, C_8-H), 11.58 (1 H, s, NH). (Found: C, 66.75; H, 4.13; N, 6.44. $C_{12}H_9NO_3$ requires: C, 66.97; H, 4.22; N, 6.51%).

(ii) *5-Acetylamino-1,2-naphthoquinone* (**44**). Recrystallization of the n-hexane insoluble part from cold acetone–n-hexane at room temp. gave 80 mg of **44** as brown needles, m.p. 157–160°. This material was very sensitive to warming. IR ν_{max}^{Nujol} cm^{-1}: 3320 (NH), 1690, 1650 (C=O). UV λ_{max}^{EtOH} mμ (log ϵ): 249.5 (4.26), 420 (3.36). NMR(DMSO-d_6) δ: 2.13 (3 H, s, $COCH_3$), 6.34 (1 H, d, J = 10.8 Hz, C_3-quinoid H), 7.50–7.79 (3 H, m, aromatic Hs), 7.74 (1 H, d, J = 10.8 Hz, C_4-quinoid H), 9.98 (1 H, br. s, NH). (Found: C, 65.36; H, 4.44; N, 6.33. $C_{12}H_9NO_3 \cdot 1/3\ H_2O$ requires: C, 65.15; H, 4.40; N, 6.33%).

Oxidation of 1-hydroxyphenanthrene (45) with Fremy's salt

The general procedure was carried out on **45**[19] (1 g), colourless needles, m.p. 157-158° (lit.[23] m.p. 155°).

(i) *1,4 - Phenanthrenequinone* (**46**). The compound **46** (499.5 mg) was obtained as yellow needles, m.p. 152–154° (lit.[23] m.p. 153°), which were recrystallized from benzene. IR ν_{max}^{Nujol} cm^{-1}: 1660 (C=O). UV λ_{max}^{EtOH} mμ (log ϵ): 224 (4.68), 262, (4.17), 279 (4.13), 289 (4.13), 370 (3.49). NMR(DMSO-d_6) δ: 7.03 (2 H, s, quinoid Hs), 7.71 (2 H, m, C_6 and C_7-H), 8.01 (2 H, d, J = 8.6 Hz, C_8 and C_9-H), 8.30 (1 H, d, J = 8.6 Hz, C_{10}–H), 9.38 (1 H, dd, J_1 = 7.0 Hz, J_2 = 2.0 Hz, C_5-H). (Found: C, 80.57; H, 3.88. $C_{14}H_8O_2$ requires: C, 80.76; H, 3.87%).

(ii) *1,2-Phenanthrenequinone* (**47**). The compound **47** (455 mg) was obtained as red needles, m.p. 202–204°, which were recrystallized from acetone. IR ν_{max}^{Nujol} cm^{-1}: 1663 (C=O). UV λ_{max}^{EtOH} mμ (log ϵ): 217.5 (4.47), 232 (4.42), 290.5 (4.31), 370 (3.38), 485 (3.00). NMR(Dioxane-d_8) δ: 6.47 (1 H, d, J = 10.5 Hz, C_3-quinoid H), 7.63 (2 H, m, aromatic Hs), 7.85–8.40 (4 H, m, aromatic Hs), 8.28 (1 H, d, J = 10.5 Hz, C_4-quinoid H). (Found: C, 80.55; H, 3.77. $C_{14}H_8O_2$ requires: C, 80.76; H, 3.87%).

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Iodinated 1,4-naphthoquinones

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Iodination of 5-amino- and 6-amino-1,4-naphthoquinones with I_2 and HIO_3 in aqueous dioxane occurs only at the benzoid ring. Depending on the reaction conditions, either aminoiodo- or aminodiiodonaphthoquinones are produced. Diazotization of these compounds followed by reduction or replacement of the diazo group with iodine affords mono- or polyiodo derivatives of 1,4-naphthoquinone.

Key words: amino-1,4-naphthoquinones, iodination, diazotization, organoiodine compounds.

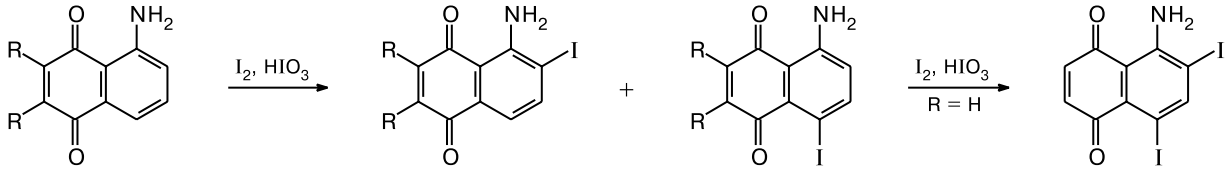
Iodo-substituted carbo- and heteroaromatic compounds serve as key intermediates in the synthesis of various acetylene, ethylene, and some functionalized aromatic derivatives.[1—3] Aryl and hetaryl iodides are often synthesized by electrophilic substitution of hydrogen.[1] However, direct iodination of polycyclic quinones (naphtho- or anthraquinone) is difficult to perform due to the deactivating effect of two electron-withdrawing carbonyl groups. In anthraquinone, this effect can be compensated by introducing the strong electron-donating amino group, which allows the amino-substituted benzoid ring to undergo iodination.[4,5] The possibility of subsequent removal and replacement of the amino group through diazonium salts provides an approach to various iodo derivatives of anthraquinone. Iodination of aminonaphthoquinones and their use as the basis for the synthesis of difficultly accessible iodo derivatives of naphthoquinone have not been described in the literature.

The direct replacement of hydrogen atoms in various aromatic substrates, including aminoanthraquinones, can be successfully performed with the use of the efficient I_2—HIO_3—H_2SO_4 iodinating system in AcOH.[4,6—8] Our attempt to use this procedure for iodination of more active aminonaphthoquinones failed due to rapid resinification of the reaction mixture. Earlier,[5] when studying iodination of aminoanthraquinones, we have proposed that H_2SO_4 should be excluded from the iodinating system by replacing it with an excess of HIO_3. This led to a substantial increase in the yield of the products and expansion of the scope of this reaction as applied to this series of compounds. In the present study, we demonstrated that the modified I_2—HIO_3 system is suitable also for iodination of aminonaphthoquinones, which allowed us to synthesize representatives of mono- and polyiodo-1,4-naphthoquinones.

Aminonaphthoquinones are iodinated at a reasonable rate in aqueous dioxane in the presence of a 5—8-fold (with respect to the stoichiometry) amount of HIO_3 at 80—87 °C. 5-Amino-1,4-naphthoquinone (**1a**) reacts with 1 equiv. of I_2 to give a mixture of isomeric 5-amino-6-iodo- (**2a**) and 5-amino-8-iodo-1,4-naphthoquinones (**3a**) (Scheme 1).

The reaction rate and the isomeric composition of the products depend on the solvent component ratio. An in-

Scheme 1



1a—c **2a—c** **3a—c** **4**

R = H (**a**), Me (**b**), Br (**c**)

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crease in the water percentage in the mixed aqueous-dioxane solvent leads to a decrease in the reaction time and a slight increase in the fraction of *para* isomer **3a** relative to *ortho* isomer **2a**. For example, an increase in dilution of dioxane with water from 7.5 : 1 to 2 : 1 leads to a decrease in the reaction time from 4 to 2.5 h, a decrease in the yield of *ortho* isomer **2a** from 54 to 23%, and an increase in the yield of *para* isomer **3a** from 22 to 29%. A decrease in the water fraction in the solvent from 1 : 7.5 to 1 : 10 leads to an increase in the reaction time to 9 h and an increase in the *ortho*-to-*para* isomer ratio from 2.4 to 2.7.

The structures of isomeric amino iodides **2a** and **3a** were proved by deamination of isomer **2a** giving rise to known 6-iodo-1,4-naphthoquinone (**5**)[9] and the transformation of isomer **3a** into 5,8-diiodo-1,4-naphthoquinone (**6**), whose structure was unambiguously established by ^{1}H NMR spectroscopy (see below).

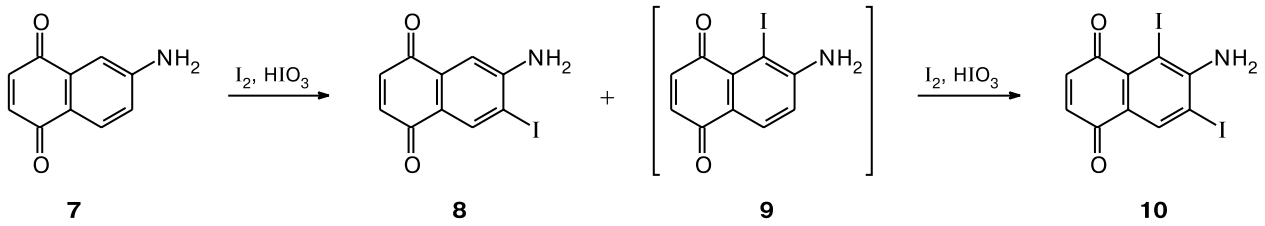
Naphthoquinones containing the substituted quinoid ring, *viz.*, 5-amino-2,3-dimethyl- (**1b**) and 5-amino-2,3-dibromo-1,4-naphthoquinone (**1c**), were also subjected to iodination under conditions favorable for the predominant formation of the *ortho* isomer (see Scheme 1). As in the case of aminoquinone **1a**, iodination of 2,3-disubstituted quinones **1b,c** occurs at the *ortho* and *para* positions with respect to the amino group. The yields of *ortho* isomers **2b,c** were 65 and 44%, respectively. The yields of *para* isomers **3b,c** were 21 and 18%, respectively. The reaction time depends substantially on the properties of the substituents in the quinoid ring of aminonaphthoquinones **1**. The presence of Me groups results in a decrease in the reaction time from 4 to 1 h. To the contrary, the presence of bromine atoms increases the reaction time to 17 h. A comparison of the ^{1}H NMR spectra of aminoiodides **2a—c** and **3a—c** revealed their characteristic features, which can be used to determine their structures (Table 1). The electron-donating amino group causes upfield shifts of the signals for the *ortho*- and *para*-protons, the shift of the signal for the *ortho*-protons being particularly large. Thus, the signal for the proton at position 6 in the spectra of 5-amino-8-iodo-1,4-naphthoquinones **3a—c** is observed at δ 6.55—6.64, whereas the signal for the proton at position 8 in the spectra of isomeric 5-amino-6-iodides **2a—c** appears at δ 7.14—7.27. The signal for the proton at position 7, which is only slightly affected by the adjacent I atom both in compounds

Table 1. Melting points, elemental analysis data, and ^{1}H NMR spectra (in $CDCl_3$) of iodinated aminonaphthoquinones **2a—c**, **3a—c**, **4**, **8**, and **10**

Compound	M.p./°C (solvent)	Found / Calculated (%)			Molecular formula	^{1}H NMR, δ (*J*/Hz)
		C	H	I		
2a	153—154 (benzene)	39.89 / 40.16	1.97 / 2.02	42.26 / 42.43	$C_{10}H_6INO_2$	6.86, 6.87 (both d, 1 H each, H(2), H(3), *J* = 10.3); 7.15 (d, 1 H, H(8), *J* = 7.9); 7.25 (br.s, 2 H, NH_2); 8.03 (d, 1 H, H(7), *J* = 7.9)
2b	170.5—171.5 (toluene—hexane)	43.86 / 44.06	3.37 / 3.08	38.89 / 38.79	$C_{12}H_{10}INO_2$	2.11, 2.13 (both s, 3 H each, 2-Me, 3-Me); 7.14 (d, 1 H, H(8), *J* = 8.0); 7.20 (br.s, 2 H, NH_2); 7.94 (d, 1 H, H(7), *J* = 8.0)
2c[a]	251—252 (toluene—hexane)	26.56 / 26.29	0.90 / 0.88	27.96 / 27.78	$C_{10}H_4Br_2INO_2$	7.27 (d, 1 H, H(8), *J* = 7.9); 7.60 (br.s, 2 H, NH_2); 8.05 (d, 1 H, H(7), *J* = 7.9)
3a	185—186 (AcOEt)	40.15 / 40.16	1.97 / 2.02	42.44 / 42.43	$C_{10}H_6INO_2$	6.61 (d, 1 H, H(6), *J* = 9.0); 6.85, 6.88 (both d, 1 H each, H(2), H(3), *J* = 10.2); 6.90 (br.s, 2 H, NH_2); 7.97 (d, 1 H, H(7), *J* = 9.0)
3b	181—182 (toluene—hexane)	44.25 / 44.06	3.27 / 3.08	38.63 / 38.79	$C_{12}H_{10}INO_2$	2.13, 2.15 (both s, 3 H each, 2-Me, 3-Me); 6.55 (d, 1 H, H(6), *J* = 8.9); 6.82 (br.s, 2 H, NH_2); 7.91 (d, 1 H, H(7), *J* = 8.9)
3c[b]	233—234 (toluene—hexane)	26.42 / 26.29	1.16 / 0.88	28.01 / 27.78	$C_{10}H_4Br_2INO_2$	6.64 (d, 1 H, H(6), *J* = 8.9); 7.00 (br.s, 2 H, NH_2); 8.00 (d, 1 H, H(7), *J* = 8.9)
4	218—219 (dichloroethane)	28.42 / 28.26	1.33 / 1.19	59.89 / 59.72	$C_{10}H_5I_2NO_2$	6.87, 6.89 (both d, 1 H each, H(2), H(3), *J* = 10.2); 7.50 (br.s, 2 H, NH_2); 8.64 (s, 1 H, H(7))
8	>210 (decomp.) (toluene)	40.36 / 40.16	2.11 / 2.02	42.39 / 42.43	$C_{10}H_6INO_2$	4.87 (br.s, 2 H, NH_2); 6.84 (s, 2 H, H(2), H(3)); 7.23 (s, 1 H, H(5)); 8.37 (s, 1 H, H(8))
10	248 (decomp.) (ethanol)	28.62 / 28.26	1.37 / 1.19	59.75 / 59.72	$C_{10}H_5I_2NO_2$	6.36 (br.s, 2 H, NH_2); 6.85 (br.s, 2 H, H(2), H(3)); 8.23 (s, 1 H, H(8))[c]

[a] Found, Br (%): 35.21. Calculated, Br (%): 34.98.
[b] Found, Br (%): 35.27. Calculated, Br (%): 34.98.
[c] In DMSO-d_6.

Scheme 2



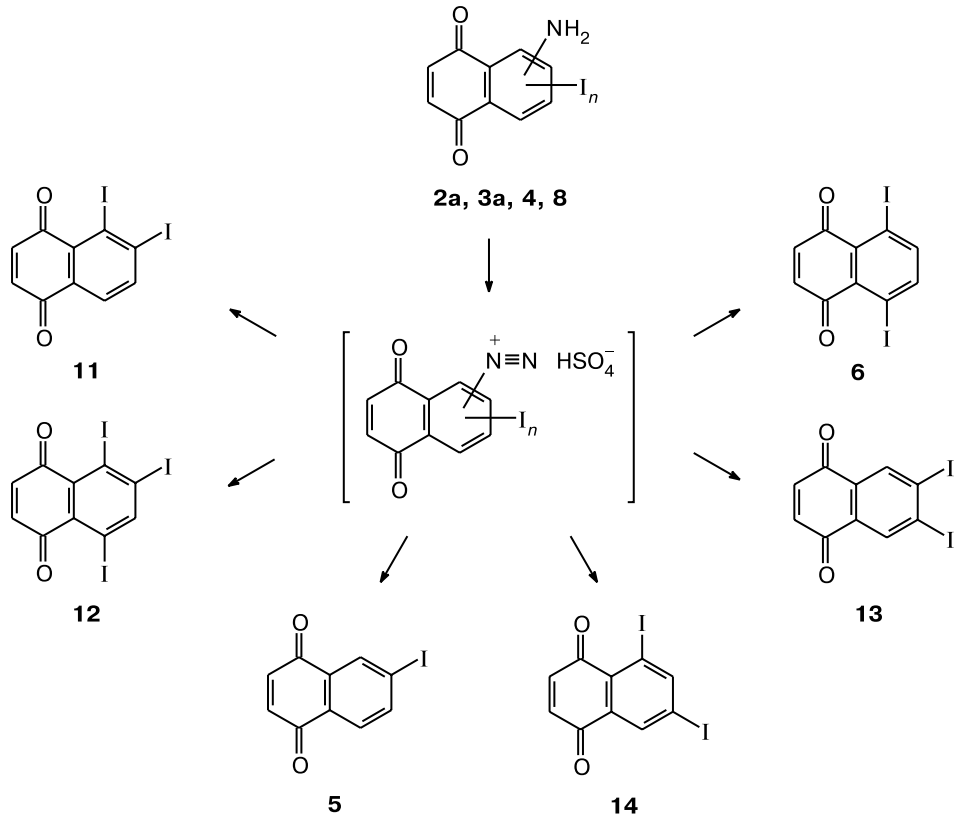
2a—c and **3a—c**, is always observed at δ 7.91—8.05. It is also noteworthy that the spin-spin coupling constant between the H(7) and H(8) protons ($J_{H(7),H(8)}$ = 8.0 Hz) is slightly smaller than that between the H(7) and H(6) protons ($J_{H(6),H(7)}$ = 9.0 Hz).

Iodination of 5-amino-1,4-naphthoquinone (**1a**) with 2 equiv. of I_2 in the presence of an excess of HIO_3 in aqueous dioxane affords 5-amino-6,8-diiodo-1,4-naphthoquinone (**4**) in 45% yield (see Scheme 1). The reaction performed with the use of dioxane and water in a ratio of 2.5 : 1 at 85 °C was completed in only 21 h. The ^{1}H NMR spectrum of diiodide **4** shows a downfield shift of a singlet for the H(7) proton under the influence of two vicinal I atoms (δ 8.64) (see Table 1).

6-Amino-1,4-naphthoquinone (**7**) is also iodinated under the above-described conditions used for iodination of 5-aminoquinone **1** (Scheme 2).

The reaction with 1 equiv. of I_2 and an excess of HIO_3 produces a mixture of 6-amino-7-iodo- (**8**) and 6-amino-5-iodo-1,4-naphthoquinone (**9**), with isomer **8** predominating (see Scheme 2). Due to substantially lower solubility in toluene, iodide **8** is easily separated from iodide **9** by crystallization. The yield of the former was 41%. Isomer **9** was not isolated in the individual state. The assignment of the structures of isomeric amino iodides **8** and **9** was made based on the ^{1}H NMR spectroscopic data. The spectrum of compound **8** shows singlets for the H(5) (δ 7.23) and H(8) protons (δ 8.37) (see Table 1), whereas the signals

Scheme 3



n = 1 (**2a**, **3a**, **8**), 2 (**4**)

for the vicinal aromatic H(7) and H(8) protons in the spectrum of compound **9** appear as doublets at δ 7.03 and 7.73, respectively. The composition of the mixture of isomeric iodides **8** and **9** generated from 6-aminoquinone **7**, unlike that prepared by iodination of 5-aminoquinone **1**, depends only slightly on the solvent component ratio. The ratio of iodides **8** and **9** changes only from 4 to 3.5 as the dioxane-to-water ratio decreases from 6.2 to 2 (^{1}H NMR spectroscopic data). The reaction of 6-amino-1,4-naphthoquinone (**7**) with 2 equiv. of I_2 and an excess of HIO_3 produced 6-amino-5,7-diiodo-1,4-naphthoquinone (**10**) in 41% yield (see Table 1).

The development of the procedure for iodination of 5-amino- and 6-aminonaphthoquinones coupled with the possibility of performing deamination and replacing the amino group with the iodine atom or other functional substituents opens the way for the synthesis of a wide range of various naphthoquinone derivatives. We used this method to synthesize a series of mono-, di-, and triiodo-substituted naphthoquinones (Scheme 3).

Diazotization of aminoiodoquinones **2a**, **3a**, **4**, and **8** was carried out with the use of $NaNO_2$ in an aqueous solution of AcOH and H_2SO_4 at 10—15 °C. The diazo group was replaced with the iodine atom by the gradual addition of a solution of the diazonium salt to an aqueous KI solution at 40—90 °C. Deamination of aminoiodoquinones **2a** and **4** was carried out by reducing the corresponding diazonium salts with H_3PO_2 at 0—5 °C. Iodonaphthoquinones **5**, **6**, and **11—14** were prepared in 37—68% yields. Their structures were completely confirmed by analytical and spectroscopic methods (Table 2).

Experimental

The ^{1}H NMR spectra were recorded on a Bruker DPX-200 instrument (200 MHz) in $CDCl_3$ at 25 °C. The progress of the reactions was monitored and the purity of the compounds were checked by TLC on Silufol UV 254 plates. 5-Amino-1,4-naphthoquinones **1a—c** were prepared according to a known procedure.[10] 6-Amino-1,4-naphthoquinone (**7**) was synthesized according to a procedure described in the study.[9] Commercial HIO_3 was used.

5-Amino-6-iodo-1,4-naphthoquinone (2a) and 5-amino-8-iodo-1,4-naphthoquinone (3a). ***A.*** Finely ground I_2 (1.95 g, 7.7 mmol) and HIO_3 (3.00 g, 17.0 mmol) in H_2O (20 mL) were successively added to a solution of 5-amino-1,4-naphthoquinone (**1a**) (3.00 g, 17.3 mmol) in dioxane (150 mL) at 70—80 °C. The reaction mixture was refluxed for 4 h, poured into water (1 L), and extracted with $CHCl_3$ (3×100 mL). The extract was washed with a 1% Na_2SO_3 solution (2×100 mL) and water. The solvent was distilled off *in vacuo* and the residue was dissolved in toluene and chromatographed on SiO_2 (200—315 μm, 40×330 mm) using the 0→4% gradient of acetone in toluene as the eluent. Amino iodide **2a** (R_f 0.15) and isomer **3a** (R_f 0.07) were prepared in yields of 2.80 g (54%) and 1.18 g (23%), respectively (see Table 1). Iodination of the same amount of aminoquinone **1a** in a mixture of dioxane (150 mL) and water (15 mL) was completed in only 9 h. The yields of *ortho* isomer **2a** and *para* isomer **3a** were 2.40 g (46%) and 0.90 g (17%), respectively.

B. Iodination of aminonaphthoquinone **1a** (1.00 g, 5.8 mmol) was performed as described above in the presence of I_2 (0.70 g, 2.8 mmol) and HIO_3 (1.70 g, 9.6 mmol) in a mixture of dioxane (50 mL) and H_2O (25 mL) with refluxing for 2.5 h. The yields of *ortho* isomer **2a** and *para* isomer **3a** were 0.40 g (23%) and 0.50 g (29%), respectively.

5-Amino-6,8-diiodo-1,4-naphthoquinone (4). A solution of amine **1a** (0.90 g, 5.2 mmol), I_2 (1.50 g, 5.9 mmol), and HIO_3 (1.80 g, 10.2 mmol) in dioxane (50 mL) and water (20 mL) was refluxed for 21 h. After chromatography on Al_2O_3 with the use of $CHCl_3$ as the eluent and recrystallization from 1,2-dichloroethane, diiodide **4** was isolated in a yield of 1.00 g (45%) (see Table 1).

5-Amino-6-iodo-2,3-dimethyl-1,4-naphthoquinone (2b) and 5-amino-8-iodo-2,3-dimethyl-1,4-naphthoquinone (3b). Iodination of 5-amino-2,3-dimethyl-1,4-naphthoquinone (**1b**) (3.50 g, 17.4 mmol) was performed analogously to aminonaphthoquinone

Table 2. Melting points, elemental analysis data, and ^{1}H NMR spectra of di- and triiodo-substituted 1,4-naphthoquinones **6** and **11—14**

Compound	M.p./°C (solvent)	Found / Calculated (%)			Molecular formula	^{1}H NMR, δ (*J*/Hz)
		C	H	I		
6	166—167 (benzene)	29.15 / 29.30	0.94 / 0.98	61.86 / 61.91	$C_{10}H_4I_2O_2$	6.98 (s, 2 H, H(2), H(3)); 7.94 (s, 2 H, H(6), H(7))
11	198—199 (benzene)	29.32 / 29.30	1.06 / 0.98	62.09 / 61.91	$C_{10}H_4I_2O_2$	6.93, 7.04 (both d, 1 H each, H(2), H(3), J = 10.2); 7.85 (d, 1 H, H(7), J = 9.0); 8.37 (d, 1 H, H(8), J = 9.0)
12	217—218 (benzene)	22.30 / 22.41	0.59 / 0.56	71.36 / 71.05	$C_{10}H_3I_3O_2$	6.97, 6.98 (both d, 1 H each, H(2), H(3), J = 10.2); 8.99 (s, 1 H, H(7))
13	232—233 (decomp.) (benzene—hexane)	29.43 / 29.30	1.11 / 0.98	61.76 / 61.91	$C_{10}H_4I_2O_2$	6.97 (s, 2 H, H(2), H(3)); 8.49 (s, 2 H, H(5), H(8))
14	178—179 (benzene—hexane)	29.48 / 29.30	1.03 / 0.98	61.72 / 61.91	$C_{10}H_4I_2O_2$	6.96, 6.98 (both d, 1 H each, H(2), H(3), J = 10.2); 8.46, 8.77 (both s, 1 H each, H(6), H(8))

1a with the use of I_2 (2.00 g, 7.9 mmol) and HIO_3 (3.05 g, 17.3 mmol) in a mixture of dioxane (150 mL) and H_2O (20 mL) with refluxing for 1 h. Chromatography on Al_2O_3 (40×110 mm) (toluene as the eluent) afforded *ortho* isomer **2b** in a yield of 3.70 g (65%) and *para* isomer **3b** in a yield of 1.20 g (21%) (see Table 1).

5-Amino-2,3-dibromo-6-iodo-1,4-naphthoquinone (2c) and 5-amino-2,3-dibromo-8-iodo-1,4-naphthoquinone (3c). A mixture of 5-amino-2,3-dibromo-1,4-naphthoquinone (**1c**) (1.00 g, 3.0 mmol), I_2 (0.35 g, 1.4 mmol), and HIO_3 (0.52 g, 2.9 mmol) in dioxane (26 mL) and H_2O (3 mL) was refluxed with stirring for 17 h. After standard work-up and separation of isomers, *ortho* isomer **2c** and *para* isomer **3c** were obtained in yields of 0.60 g (44%) and 0.25 g (18%), respectively (see Table 1).

6-Amino-7-iodo-1,4-naphthoquinone (8). 6-Amino-1,4-naphthoquinone (**7**) (1.00 g, 5.8 mmol), I_2 (0.65 g, 2.6 mmol), and HIO_3 (1.00 g, 5.7 mmol) in a mixture of dioxane (50 mL) and water (25 mL) were refluxed for 1 h. After standard isolation, the product was dissolved in a toluene—$CHCl_3$ mixture and filtered through an Al_2O_3 layer. Then the solvent was removed *in vacuo*, and the residue (1.00 g) consisting of 6-amino-7-iodo- (**8**) and 6-amino-5-iodo-1,4-naphthoquinone (**9**) in a ratio of 4 : 1 (^{1}H NMR spectroscopic data) was recrystallized from toluene. Iodide **8** was isolated in a yield of 0.70 g (41%) (see Table 1).

6-Amino-5,7-diiodo-1,4-naphthoquinone (10). A solution of 6-amino-1,4-naphthoquinone (**7**) (0.90 g, 5.2 mmol), I_2 (1.50 g, 5.9 mmol), and HIO_3 (1.80 g, 10.2 mmol) in dioxane (50 mL) and water (25 mL) was refluxed for 1 h. Diiodide **10** was obtained in a yield of 0.91 g (41%) (see Table 1).

5,7-Diiodo-1,4-naphthoquinone (14). A solution of $NaNO_2$ (0.32 g, 4.5 mmol) in water (4 mL) was added to a solution of 5-amino-6,8-diiodo-1,4-naphthoquinone (**4**) (0.88 g, 2.1 mmol) in a mixture of AcOH (15 mL), concentrated H_2SO_4 (27 mL), and water (10 mL) at 10 °C. The reaction mixture was stirred at this temperature for 30 min. The resulting solution of the diazonium salt was carefully poured into a mixture of 48% H_3PO_2 (11 mL) and ice (200 g), and the mixture was allowed to stand for ~14 h. The precipitate that formed was filtered off, washed with water, and dried. Chromatography on Al_2O_3 (benzene and $CHCl_3$ as the eluents) afforded diiodide **14** in a yield of 0.44 g (52%) (see Table 2).

6-Iodo-1,4-naphthoquinone (5). Deamination of 5-amino-6-iodo-1,4-naphthoquinone (**2a**) (1.0 g, 3.3 mmol) was performed under the same conditions as amine **4**. Compound **5** was obtained in a yield of 0.35 g (37%), m.p. 123—124 °C (*cf.* lit. data[9]).

5,6-Diiodo-1,4-naphthoquinone (11). A solution of $NaNO_2$ (0.60 g, 8.5 mmol) in water (1 mL) and 60% H_2SO_4 (3.5 mL) were added to a solution of 5-amino-6-iodo-1,4-naphthoquinone (**2a**) (1.50 g, 5.0 mmol) in AcOH (36 mL) at 15 °C. The reaction mixture was cooled to 10 °C. Then 10% H_2SO_4 (35 mL) was added and the mixture was stirred for 30 min. The resulting solution of the diazonium salt was added with vigorous stirring to a solution of KI (2.80 g, 16.9 mmol) in water (180 mL) at 40 °C. The precipitate that formed was filtered off, washed with water, dried, and chromatographed on SiO_2 using benzene as the eluent. The yield of diiodide **11** was 1.40 g (68%) (see Table 2).

5,8-Diiodo-1,4-naphthoquinone (6). Diazotization of 5-amino-8-iodo-1,4-naphthoquinone (**3a**) (0.80 g, 2.7 mmol) was performed as described above in the synthesis of diiodide **11**. The resulting solution of the diazonium salt was added to a stirred solution of KI (1.50 g, 9.0 mmol) in water (60 mL) preheated to 90 °C. The resulting suspension was heated to boiling and then cooled. The precipitate that formed was filtered off, washed with water, dried, and chromatographed on Al_2O_3 using 1,2-dichloroethane as the eluent. The yield of diiodide **6** was 0.50 g (46%) (see Table 2).

6,7-Diiodo-1,4-naphthoquinone (13). A solution of $NaNO_2$ (0.33 g, 4.6 mmol) in water (3 mL) and 50% H_2SO_4 (8 mL) were gradually added to a solution of aminonaphthoquinone **8** (0.90 g, 3.0 mmol) in AcOH (16 mL) at 10 °C. The reaction mixture was stirred at this temperature for 1 h. The resulting solution of the diazonium salt was poured into a stirred mixture of KI (2.90 g, 17.5 mmol) in water (50 mL) and toluene (50 mL), which was preheated to 60 °C. After cooling, the toluene layer was separated, and the aqueous layer was extracted with toluene. The combined extracts were washed with an aqueous Na_2CO_3 solution and water and filtered through an Al_2O_3 layer. Diiodide **13** was obtained in a yield of 0.80 g (65%) (see Table 2).

5,6,8-Triiodo-1,4-naphthoquinone (12). Initially, 5-amino-6,8-diiodo-1,4-naphthoquinone (**4**) (0.50 g, 1.2 mmol) was subjected to diazotization, and then the diazo group was replaced with iodine, as described above in the synthesis of diiodide **11**. After chromatography on SiO_2 with the use of 1,2-dichloroethane as the eluent, triiodide **12** was isolated in a yield of 0.26 g (41%) (see Table 2).

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Overcoming naphthoquinone deactivation: rhodium-catalyzed C-5 selective C–H iodination as a gateway to functionalized derivatives†

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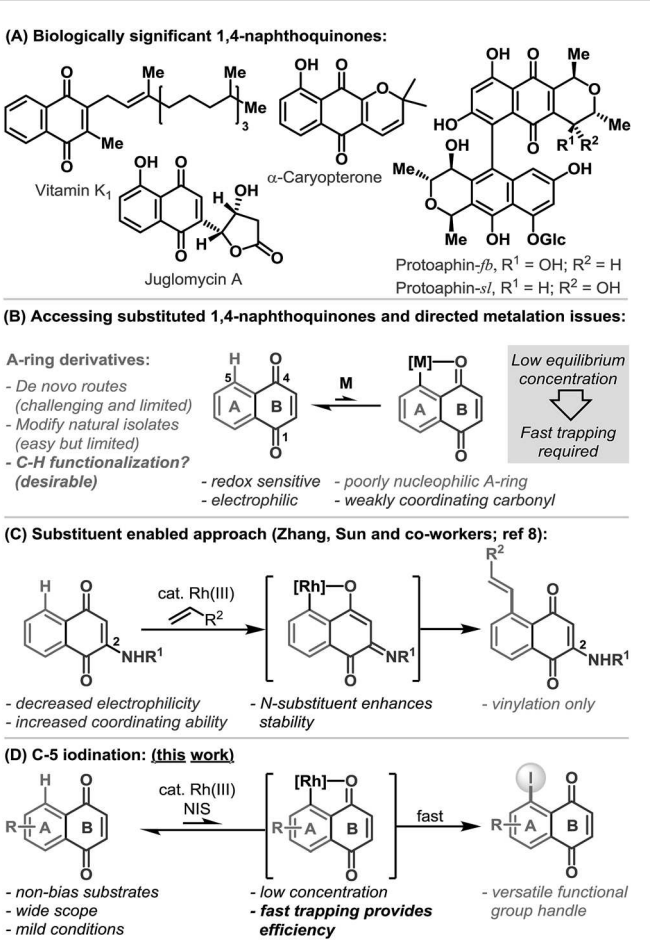
We report a Rh-catalyzed method for the C-5 selective C–H iodination of naphthoquinones and show that complementary C-2 selective processes can be achieved under related conditions. C–C bond forming derivatizations of the C-5 iodinated products provide a gateway to previously inaccessible A-ring analogues. The present study encompasses the first catalytic directed *ortho*-functionalizations of simple (non-bias) naphthoquinones. The strategic considerations outlined here are likely to be applicable to C–H functionalizations of other weakly coordinating and/or redox sensitive substrates.

Introduction

1,4-Naphthoquinones function as redox cyclers and alkylating agents in a wide range of biological processes.[1] For example, vitamin K encompasses a family of 2-methyl-1,4-naphthoquinones that act as cofactors for the post-translational carboxylation of glutamic acid residues, a process that is essential to blood coagulation and bone metabolism.[1e] Other notable naphthoquinones include the juglomycins,[2a] dimeric pyranonaphthoquinones, such as protoaphin-fb and protoaphin-sl[2b–e] and the lapachones (Scheme 1A).[2f] Because of their biological importance, significant efforts are devoted to the synthesis and evaluation of a wide range of naphthoquinone derivatives.[3] While methods for the modification of the quinone B-ring are reasonably well established,[4] flexible protocols that allow the direct functionalization of the benzenoid A-ring are rare (Scheme 1B).[5] This situation is exacerbated by the limitations associated with *de novo* naphthoquinone construction.[6] Consequently, medicinal studies on A-ring analogues are, in the main, limited to simple derivatives of natural isolates.

Catalytic directed *ortho*-C–H metalation has emerged as a powerful platform for the preparation of diverse aromatic compounds.[7] However, its application to the modification of naphthoquinones is limited by (a) their susceptibility to reduction, (b) their high electrophilicity, (c) the low nucleophilicity of the benzenoid A-ring and (d) the weak coordinating ability of the B-ring carbonyls (Scheme 1B). Indeed, we are aware of only one protocol that enables catalytic carbonyl directed C–H functionalization of the naphthoquinone A-ring



Scheme 1

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† Electronic supplementary information (ESI) available: Experimental procedures and characterisation data for all compounds are provided. CCDC 1441683–1441694. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6sc00302h

(Scheme 1C).[8] Here, Rh(III)-catalyzed C-5 vinylation required the strategic installation of a C-2 amino substituent, which was crucial for facilitating cyclometalation.

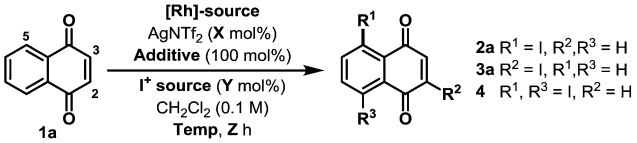
Carbonyl directed *ortho*-metalation with late transition metals is usually a reversible process,[7,9] where the equilibrium depends upon both the nucleophilicity of the arene and the coordinating strength of the directing group (Scheme 1B). Neither aspect is favorable for naphthoquinones, so an efficient process must necessarily rely upon fast trapping of the metalated intermediate. In considering this, we sought to introduce a synthetically versatile handle under conditions that avoid nucleophilic or reducing reagents. Consequently, we were drawn to the *ortho*-C–H iodination protocol reported by Glorius and co-workers,[9] which involves the trapping of cyclometalated aryl-Rh(III) complexes with NIS. This reagent is highly reactive, oxidative and also non-nucleophilic, such that it seemed well suited to C-5 selective iodination. In this report, we outline the development and scope of this protocol, which provides, for the first time, a C–H activation-based gateway to diverse A-ring analogues (Scheme 1D). Additionally, we disclose that, in certain cases, fine tuning of the Rh-system enables a complete switch to C-2 selective iodination.

Results and discussion

Preliminary studies involved exposing naphthoquinone **1a** to a variety of electrophilic iodine sources in the presence of *in situ* generated cationic Rh(III)-systems (Table 1). These experiments revealed that achieving both high conversion and high C-5 regioselectivity was likely to be challenging. $[RhCp^*Cl_2]_2/AgNTf_2$ in combination with NIS and $Cu(OAc)_2$ resulted in only a 39% yield of **2a**, albeit with 10 : 1 selectivity over C-2 regioisomer **3a** (entry 1). Other electrophilic iodine sources were less effective; for example, DIH led to substantial quantities of C-2 adduct **3a** and bis-iodinated product **4** (entry 2). Extensive optimization efforts were undertaken to identify an effective system, and, in part, these studies focussed on the use of other acetate ligated Lewis acids in combination with a range of Rh(III)-precatalysts (entries 3–8).[10] However none of these systems were especially effective, with the key issue being competitive degradation of the iodinating agent under the reaction conditions. To resolve this, microwave conditions were investigated.[11] Pleasingly, by heating at 65 W (50 °C), a 54% yield of **2a** was obtained after just 2.5 hours (entry 9). Under these conditions, the major byproduct was bis-iodinated adduct **4**, which was formed in 14% yield. Further refinement led to the conditions outlined in entry 10, which deliver **2a** in 69% yield and with high selectivity over **3a** and **4**.[12] The conversion of **1a** to **2a** has been achieved previously in 34% overall yield, but this required a substrate specific 5 step sequence *via* a potentially hazardous diazonium intermediate.[13] Thus, the efficiency and generality (*vide infra*) of the new protocol described here is both notable and synthetically enabling.

The scope of the new process is outlined in Table 2. Naphthoquinones **1b–e** possessing C-8 substitution underwent efficient iodination to provide targets **2b–e** in good to excellent yield. As expected, the efficiency of the process decreases as the benzenoid ring becomes more electron deficient, and, accordingly, nitro adduct **2f** was generated in only 24% yield. Systems with substituents at C-6 or C-7 can potentially deliver two different regioisomeric products. Perhaps as a result of secondary coordination by the methoxy group, naphthoquinone **1g** afforded selectively adduct **2g**, wherein iodination has occurred at the more hindered *ortho*-position. Conversely, methyl-substituted system **1h** favored iodination at the less hindered *ortho*-site to deliver iodide **2h** in 63% yield. Halogen substituents are tolerated and bromo- and chloro-naphthoquinones **1j–l** were converted to targets **2j–l** in synthetically useful yields. For **2j**, the high *ortho*-regioselectivity may reflect

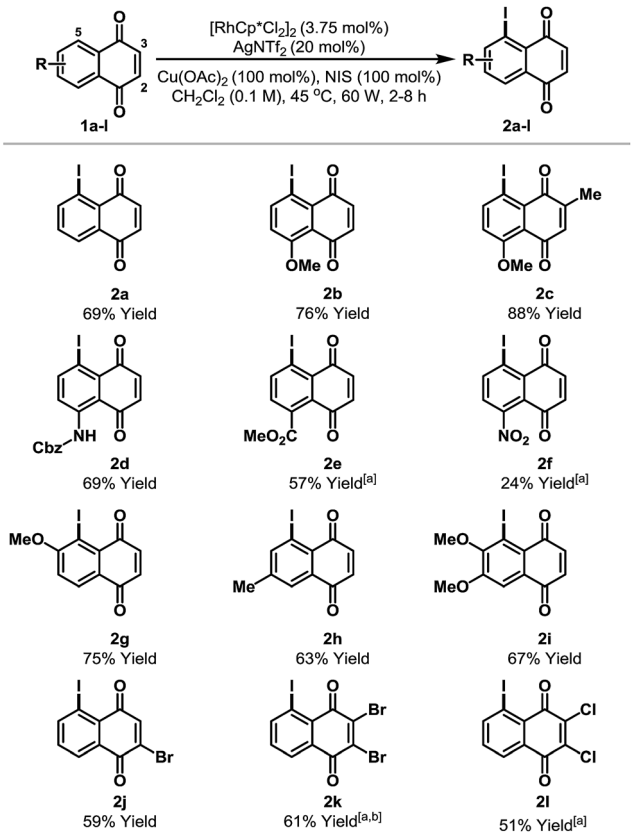
Table 1 Selected optimization results



Entry	Rh-source	*X*	Additive	I^+ source	*Y*	Temp/°C	*Z*	**2a : 3a : 4**
1	$[RhCp^*Cl_2]_2$ (2.5%)	10	$Cu(OAc)_2$	NIS	220	120	22	39 : 8 : 0
2	$[RhCp^*Cl_2]_2$ (2.5%)	10	$Cu(OAc)_2$	DIH	140	120	22	4 : 14 : 10
3	$[RhCp^*Cl_2]_2$ (2.5%)	10	$Cu(OPiv)_2$	NIS	140	120	22	14 : 0 : 0
4	$[RhCp^*Cl_2]_2$ (2.5%)	10	$Zn(OAc)_2$	NIS	140	120	22	18 : 0 : 0
5	$[RhCp^*Cl_2]_2$ (4%)	10	$Cu(OAc)_2$	NIS	120	100	16	44 : 5 : 0
6	$[RhCp^{*i\text{-}Pr}Cl_2]_2$ (4%)	10	$Cu(OAc)_2$	NIS	120	100	16	39 : 8 : 0
7	$[RhCp^{*CF_3}Cl_2]_2$ (4%)	10	$Cu(OAc)_2$	NIS	120	100	16	3 : 0 : 0
8	$[RhCp^*(OAc)_2]$ (4%)	10	$Cu(OAc)_2$	NIS	120	100	16	32 : 2 : 0
9	$[RhCp^*Cl_2]_2$ (4%)	10	$Cu(OAc)_2$	NIS	120 50[a]	(65 W)	2.5	54 : 0 : 14
10	$[RhCp^*Cl_2]_2$ (3.75%)	20	$Cu(OAc)_2$	NIS	100 45[a]	(60 W)	2	69 : 0 : 5

[a] External temperature of the reaction vessel. NIS = *N*-iodosuccinimide. DIH = 1,3-diiodo-5,5-dimethylhydantoin. $Cp^{*i\text{-}Pr}$ = isopropyl tetramethylcyclopentadienyl. Cp^{*CF_3} = trifluoromethyl tetramethylcyclopentadienyl.

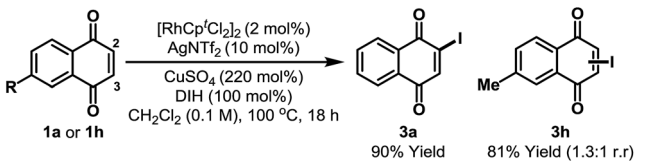
Table 2 Scope of the C-5 selective iodination protocol[a]



[a] <5% bis-iodination was observed in all cases. NIS = *N*-iodosuccinimide. [a] 5 mol% $[RhCp^*Cl_2]_2$ and 27 mol% $AgNTf_2$ were used. [b] Run at 65 °C and 75 W.

the higher basicity of the C-4 carbonyl of **1j**. In all cases, only trace quantities (≤5%) of bis-iodinated and C-2/3 iodinated products were observed (*cf.* **4** and **3a**), and no significant iodination occurred in the absence of Rh-catalyst. Structural assignments were based on detailed NMR analysis (DEPT, COSY, HSQC, HMBC) and X-ray structures of **2a–c**, **2f**, **2j** and **2k**.[14]

During optimization we noted that the regioselectivity of iodination is strongly influenced by the nature of the Lewis acidic additive. Acetate based systems consistently provided high selectivity for C-5, likely *via* a Rh-acetate promoted concerted metalation–deprotonation mechanism (*cf.* Table 1, entry 8).[15] By switching from $Cu(OAc)_2$ to $CuSO_4$ we were able to develop a complementary C-2 selective iodination protocol (Scheme 2).[10,16] Under optimized conditions, iodination of **1a** generated **3a** in 90% yield and with complete regioselectivity. Perez and co-workers have shown that morpholine–iodine complex can convert **1a** to **3a**, but in only 35% yield and as a mixture of products.[17] For unsymmetrical systems, such as **1h**, C-2 *vs.* C-3 selectivity was not readily controlled and **3h** was formed as a mixture of these two regioisomers.[18] At the present stage, C-3 selective iodination of systems possessing substitution at C-2 is not feasible, perhaps due to steric inhibition of the C–H metalation event.



Scheme 2 C-2 selective iodination. DIH = 1,3-diiodo-5,5-dimethylhydantoin. Cp^t = 1,3-di-*tert*-butylcyclopentadienyl.

In principle, the activation mode employed here should enable other selective naphthoquinone C–H functionalizations. However, as outlined in the introduction, efficient processes likely require highly reactive and non-reducing coupling partners. Accordingly, we have been unable to achieve direct C–H activation based C–C bond formations.[19] However, the iodinated products described here provide a gateway to this important goal (Fig. 1). Because of the synthetic inaccessibility of A-ring halogenated naphthoquinones, Pd-catalyzed cross-couplings involving the benzenoid ring have not been developed. This aspect is challenging because the quinone moiety can act as an oxidant or ligand for Pd.[20] For example, arylation of **2a** could not be achieved under Suzuki conditions and only decomposition products were observed. After extensive investigation, we established that mild Stille cross-couplings[21] are effective and, using this approach, arylated derivative **5a** was isolated in high yield. Heck reactions are another promising avenue and Pd(0)-catalyzed reaction of **2a** with ethyl acrylate delivered **5b** in 66% yield.[22] To date, alkynylation under Sonogashira conditions has not been fruitful but the use of stoichiometric alkynyl copper(I) reagents is feasible and this allowed the isolation of **5c** in 64% yield.[13] The studies outlined in Fig. 1 validate short and diversifiable entries to previously challenging naphthoquinone targets.

Preliminary results suggest that other highly electrophilic reagents might also be effective for C-5 selective functionalization (Scheme 3). Using DBH, Rh-catalyzed C-5 selective bromination of **1a** proceeded in 66% yield to afford a 7 : 2 mixture of C-5 and C-2 bromides **6**/**7**; the structure of **6** was confirmed by single crystal X-ray diffraction.[13] The most direct previous entry to **6** involved oxidation of 2-bromonaphthalene, but this afforded the target in only 15% yield and as a complex mixture of isomers.[23] Alternative C-2 selective bromination can be achieved by adapting the conditions outlined in Scheme 2 and this enabled the selective formation of **7** in 88% yield from **1a**.

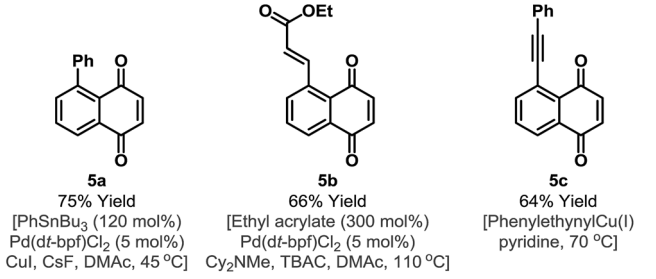
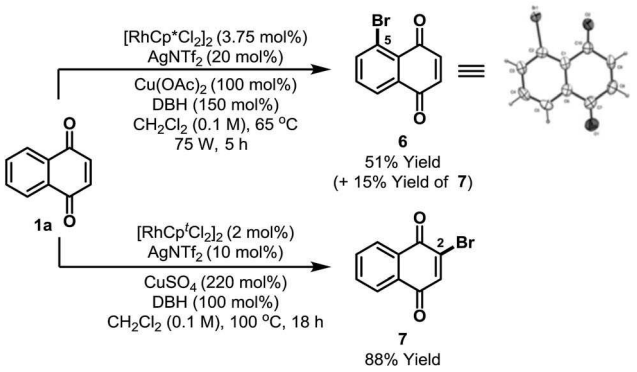


Fig. 1 C–C bond forming derivatizations of **2a**. TBAC = tetra-*n*-butylammonium chloride, DMAc = *N*,*N*-dimethylacetamide.



Scheme 3 C-5 and C-2 selective bromination. DBH = 1,3-dibromo-5,5-dimethylhydantoin. Cp^t = 1,3-di-*tert*-butylcyclopentadienyl.

Conclusions

In conclusion, we report an efficient and reliable methodology for C-5 selective C–H iodination of naphthoquinoidal compounds and show that complementary C-2 selective processes can be achieved under related conditions. To the best of our knowledge, the present study provides the first method for catalytic directed *ortho*-functionalization of simple (non-bias) naphthoquinones. The iodinated products are amenable to C–C bond forming derivatizations and this enables flexible modifications to the naphthoquinone A-ring. The chemistry opens up new avenues for biological investigation and is likely to be of wide general interest. In broader terms, the strategic considerations outlined here may guide the development of catalytic C–H functionalizations involving other weakly coordinating and/or redox sensitive substrates.

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Synthesis of substituted benz[*g*]indole-6,9-diones and benzo[*h*]quinoline-7,10-diones by heterocyclization of 6-alkynyl-5-amino-1,4-naphthoquinones

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Substituted benz[*g*]indole-6,9-diones were synthesized by intramolecular cyclization of 6-alkynyl-5-amino-3-diethylamino-1,4-naphthoquinones. A method was developed for the preparation of 2-aryl(or alkyl)-4,9-bis(dialkylamino)benzo[*h*]quinoline-7,10-diones, which involves the addition of a secondary amine to 6-acylethynyl-5-amino-3-diethylamino-1,4-naphthoquinone followed by cyclization of the resulting adduct.

Key words: 6-alkynyl-5-amino-3-diethylamino-1,4-naphthoquinones, heterocyclization, benz[*g*]indole-6,9-diones, 2-aryl(or alkyl)-4,9-bis(dialkylamino)benzo[*h*]quinoline-7,10-diones.

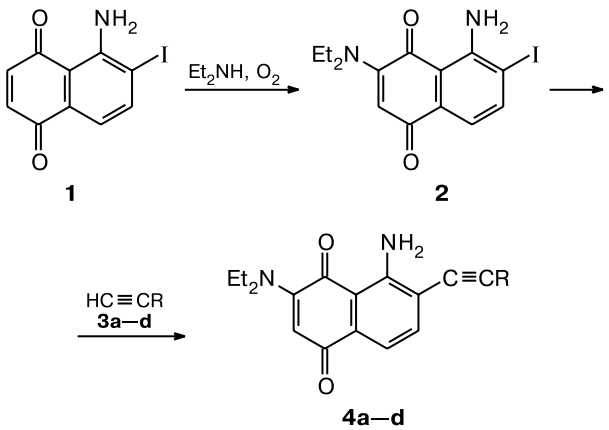
High biological activity of many fused heterocyclic quinone derivatives constantly stimulates researchers to develop procedures for the synthesis of new compounds of this class, study their chemical transformations, and search for new promising pharmacophoric structures.[1—3]

Earlier,[4—8] we have synthesized various N-, O-, and S-containing heterocycles fused to quinones with the use of alkynyl derivatives of anthraquinone as the key precursors. The procedures for the preparation of alkynyl derivatives of naphthoquinone are poorly developed, and these compounds have been used for this purpose only in rare cases.[9—11] It should be noted that heterocyclization of alkynylnaphthoquinones often follows a pathway different from that observed for the analogous reactions of anthraquinone derivatives.[10,12,13] Hence, methods of heterocyclization of alkynylanthraquinones cannot *a priori* be extended to the synthesis of naphthoquinone derivatives.

In the present study, we propose a method for synthesizing previously unknown heterocyclic systems fused to quinones, *viz.*, benz[*g*]indole-6,9-diones and benzo[*h*]quinoline-7,10-diones, by cyclization of 6-alkynyl-5-aminonaphthoquinones.

To prepare the key alkynyl compounds, the starting 5-amino-6-iodo-1,4-naphthoquinone (**1**) was subjected to oxidative amination with diethylamine (Scheme 1). Due to the strong orientation effect of the amino group at position 5,[14] the diethylamino group was directed predominantly at position 3. The yield of 5-amino-3-diethylamino-6-iodonaphthoquinone (**2**) was 89%. The corresponding 2-diethylamino isomer was formed in minor amount. It was necessary to block the quinonoid ring to avoid competitive reactions with nucleophiles in subsequent steps. Condensation of iodide **2** with terminal acetylenes **3a—d** was carried out in aqueous dioxane in the presence of $Pd(PPh_3)_2Cl_2$, CuI, and Na_2CO_3. This modification of cross-coupling, which has been initially proposed for iodoanthraquinones,[15] proved to be also applicable to iodonaphthoquinones containing the protected quinonoid ring.[13] 6-Alkynyl-5-amino-3-diethylaminonaphthoquinones **4a—d** were prepared in 80—95% yields.

Scheme 1



4a—d

R = Ph (**a**), C(OH)Me_2 (**b**), CH(OH)Ph (**c**), CH(OH)$CHMe_2$ (**d**)

The possibility of the pyrrole ring closure in *vic*-(alkynyl)aminonaphthoquinones **4** giving rise to sub-

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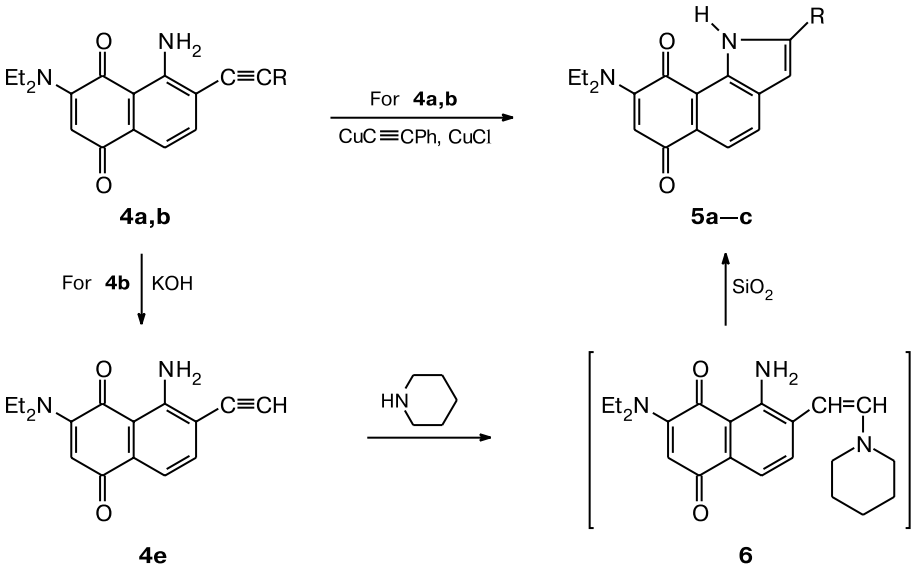
1066-5285/05/5402-0421

Scheme 2



R = Ph (**4a**, **5a**), C(OH)Me_2 (**4b**), H (**4e**, **5b**), C$Me{=}CH_2$ (**5c**)

stituted benz[*g*]indole-6,9-diones **5** was exemplified by cyclization of phenylethynyl derivative **4a**, tertiary acetylenic alcohol **4b**, and ethynyl derivative **4e** (Scheme 2). Compound **4e** was prepared by alkaline cleavage of alcohol **4b** (the retro-Favorskii reaction).[5]

Aminoacetylenes **4a,b** were subjected to cyclization in DMF in the presence of PhC≡CCu and CuCl[16] at 155 °C for 4.5—5 h. The yield of 2-phenylbenzindoledione **5a** was as high as 83%. Cyclization of alcohol **4b** was accompanied by dehydration and gave isopropenylbenzindoledione **5c** instead of the expected 2-(1-hydroxy-1-methylethyl)-8-diethylaminobenz[*g*]indole-6,9-dione as the major product (55% yield).

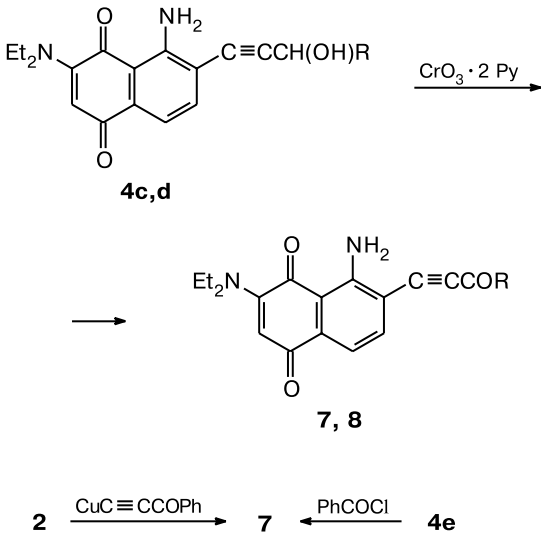
Earlier,[16,17] it has been demonstrated that the pyrrole ring closure in *vic*-(alkynyl)aminoanthraquinones can be performed in the absence of Cu^I compounds and, what is more important, under much milder conditions. This process involves the nucleophilic addition of a secondary amine at the triple bond, which is highly electrophilic in these compounds, followed by cyclization of the adduct in the presence of mineral acids or on SiO_2.

We extended this method to naphthoquinone derivatives. The addition of piperidine to ethynylnaphthoquinone **4e** occurred at 70 °C during 3.5 h to give adduct **6**. Being adsorbed on SiO_2 in $CHCl_3$, adduct **6** underwent cyclization with elimination of piperidine to form 8-diethylaminobenz[*g*]indole-6,9-dione (**5b**) in 80% yield.

Thus, cross-coupling of 5-amino-3-diethylamino-6-iodonaphthoquinone with terminal acetylenes followed by heterocyclization of the resulting acetylenic derivatives provides a convenient route to substituted benz[*g*]indole-6,9-diones.

In intramolecular cyclization giving rise to six-membered heterocycles, *vic*-functionalized acylethynyl derivatives can serve as precursors.[6,7,18] Hence, to prepare naphthoquinones angularly annelated to the pyridine ring, we oxidized secondary alcohols **4c,d** with the Collins reagent at 20 °C to ketones **7** and **8** (Scheme 3) in 94 and 78% yields, respectively.

Scheme 3



R = Ph (**4c**, **7**), CHMe_2 (**4d**, **8**)

In addition, ketone **7** was synthesized by condensation of iodide **2** with cuprous benzoylacetylide[19] and by

benzoylation of ethynylnaphthoquinone **4e**.[20] Unfortunately, the acetylide method is applicable only in certain cases because of instability of many acylacetylides. As for catalytic acylation, it is complicated by side reactions and gives products in low yields.

The simplest procedure for the closure of the nitrogen-containing six-membered ring in such compounds, including anthraquinones, is based on cyclization under conditions of hydration of the triple bond.[21]

We attempted to perform cyclization of ketone **7** to the corresponding benzo[*h*]quinolinetrione. To avoid hydrolysis of the diethylamino group, one had to carry out the reaction in dioxane in the presence of $HgSO_4$ and small amounts of 45% H_2SO_4 (1.5% v/v). However, even under these conditions, only hydrolysis of compound **7** occurred to give 5-amino-6-benzoylethynyl-3-hydroxy-1,4-naphthoquinone (**9**), whereas no heterocyclic ring closure was observed. We failed to perform cyclization of hydroxynaphthoquinone **9** by increasing the H_2SO_4 concentration and changing the amount of the mercury catalyst.

A more efficient approach to the construction of the benzo[*h*]quinoline system based on ketones **7** and **8** involves cyclization of aminovinyl ketones, which are prepared by the addition of amines to acetylenic ketones.[18] The formation of adducts leads to changes in the geometry of the unsaturated substituent and, as a consequence, to a decrease in the distance between the reaction centers necessary for the heterocyclic ring closure.

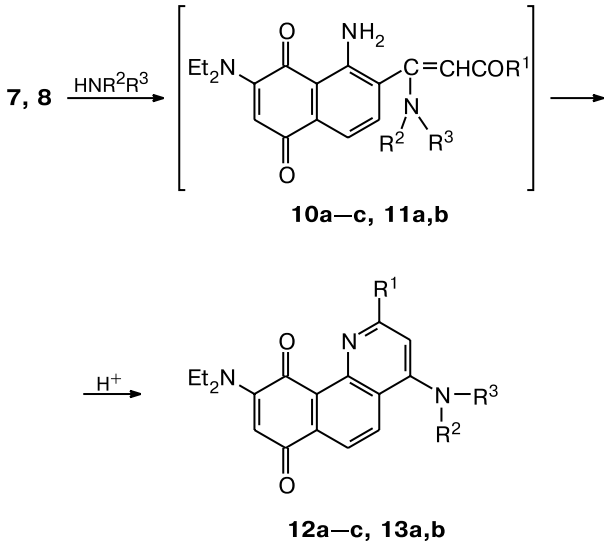
The reactions of acetylenic ketones **7** and **8** with an excess of amine at 20 °C afforded adducts **10a—c** and **11a,b**, which were subjected to cyclization in a biphasic system benzene—12% HCl (Scheme 4).

The yields of substituted benzo[*h*]quinoline-7,10-diones **12a—c** and **13a,b** were 50—90%. No complications associated with hydrolysis of the diethylamino group were observed.

Thus, the method of the construction of the 4-amino-substituted pyridine ring, which involves the vicinal amino- and acylethynyl groups and proceeds *via* the aminovinyl adducts, was extended to naphthoquinone derivatives. This made it possible to prepare substituted benzo[*h*]quinoline-7,10-diones, thus extending the range of compounds, which, like related anthraquinone derivatives, serve as chelating and potentially biologically active compounds.[22,23]

The characteristic differences observed in the ^{1}H NMR spectra of alkynylnaphthoquinones **4**, **7**, and **8**, benz[*g*]indole-6,9-diones **5**, and benzo[*h*]quinoline-7,10-diones **12** and **13** can be used to make assignments of the structures (Tables 1 and 2). The chemical shifts of the protons of the benzenoid ring slightly increase in the series **4**, **7**, **8** → **5** → **12**, **13** (δ 7.3—7.8, 7.8—7.9, and 8.0—8.4, respectively). The involvement of the N atom of the primary amino groups in acetylenic compounds **4**, **7**, and **8** in ring systems brings about a downfield shift of the signal for the proton in the *para* position of the benzenoid ring. The formation of the indole structure leads to the shift of ~0.4—0.6 ppm (for the quinoline structure, the shift is no smaller than 0.6 ppm). It should be noted that the character of substitution in the benzenoid ring is poorly reflected in the chemical shifts of the proton of the quinonoid ring (δ 5.71—5.85). The replacement of the diethylamino group with the hydroxy group leads to an upfield shift of this proton by ~0.5 ppm. The introduction of the phenyl substituent at position 2 of the heterocycle of the benzindoledione and benzoquinolinedione systems causes a downfield shift of the signal for the adjacent proton by ~0.3 and ~0.6 ppm, respectively. The nature of the dialkylamino group at position 4 of quinolines **12** and **13** has virtually no effect on the chemical shift of the proton at position 3.

Scheme 4



R^1 = Ph (**7**, **10**, **12**), $CHMe_2$ (**8**, **11**, **13**);
$R^2 + R^3 = (CH_2)_5$ (**a**), $(CH_2)_2O(CH_2)_2$ (**b**); $R^2 = R^3$ = Et (**c**)

Experimental

The ^{1}H NMR spectra were recorded on a Bruker DPX-200 instrument (200 MHz) in $CDCl_3$ at 25 °C. The IR spectra were measured on a UR-20 spectrometer in $CHCl_3$. The UV spectra were recorded on a Shimadzu 2401PC spectrometer in benzene. The reactions were monitored and the purity of the reaction products was checked by TLC on Silufol UV 254 plates. Compounds **3** were commercial reagents.

5-Amino-3-diethylamino-6-iodo-1,4-naphthoquinone (2). Diethylamine (20 mL) was added with stirring to a solution of iodide **1** (2.60 g, 8.7 mmol) and $Cu(OAc)_2 \cdot H_2O$ (0.60 g, 3.0 mmol) in dioxane (70 mL) for 15 min. Then air was passed through the solution at 20 °C for 2 h. The reaction mixture was poured into water (0.5 L) and extracted with $CHCl_3$ (3×100 mL).

Table 1. Melting points, results of elemental analysis, and ^{1}H NMR and IR spectra of acetylenic derivatives of naphthoquinones **4a—e** and **7—9**

Compound	M.p./°C (toluene—hexane)	Found / Calculated (%)			Molecular formula	^{1}H NMR, δ (*J*/Hz)	IR, ν/cm^{-1}
		C	H	N			
4a	87—88	76.65 76.72	5.68 5.85	8.00 8.13	$C_{22}H_{20}N_2O_2$	1.29 (t, 6 H, 2 Me, *J* = 7.0); 3.52 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.84 (s, 1 H, H(2)); 7.37 (d, 1 H, H(8), *J* = 7.7); 7.62 (d, 1 H, H(7), *J* = 7.7); 7.30—7.45 (m, 3 H, *m*-H arom., *p*-H arom.); 7.45—7.60 (m, 4 H, 2 *o*-H arom. + NH_2)	1620, 1645 (C=O); 2220 (C≡C); 3360, 3485 (NH_2)
4b	85—86	70.04 69.92	6.98 7.02	8.75 8.58	$C_{19}H_{22}N_2O_3$	1.28 (t, 6 H, 2 NCMe, *J* = 7.2); 1.67 (s, 6 H, 2 Me); 2.35 (br.s, 1 H, OH); 3.48 (q, 4 H, 2 NCH_2, *J* = 7.2); 5.78 (s, 1 H, H(2)); 7.00 (br.s, 2 H, NH_2); 7.27 (d, 1 H, H(8), *J* = 7.6); 7.42 (d, 1 H, H(7), *J* = 7.6)	1620, 1650 (C=O); 2220 (C≡C); 3360, 3485 (NH_2); 3620 (OH)
4c	109—110	73.59 73.78	6.18 5.92	7.46 7.48	$C_{23}H_{22}N_2O_3$	1.27 (t, 6 H, 2 Me, *J* = 7.0); 3.51 (q, 4 H, 2 NCH_2, *J* = 7.0); 2.85 (br.s, 1 H, OH); 5.82 (s, 1 H, H(2)); 5.79 (d, 1 H, CHO, *J* = 8.0); 7.34 (d, 1 H, H(8), *J* = 7.7); 7.54 (d, 1 H, H(7), *J* = 7.7); 7.35—7.50 (m, 3 H, *m*-H arom., *p*-H arom.); 7.55—7.65 (m, 2 H, *o*-H arom.); 7.00 (br.s, 2 H, NH_2)	1620, 1645 (C=O); 2220 (C≡C); 3365, 3485 (NH_2); 3600 (OH)
4d	106—107	70.54 70.56	7.21 7.11	8.08 8.23	$C_{20}H_{24}N_2O_3$	1.27 (t, 6 H, 2 NCMe, *J* = 6.8); 1.90—2.20 (m, 1 H, CH); 1.06, 1.08 (both d, 3 H each, 2 MeC, *J* = 6.3); 3.49 (q, 4 H, 2 NCH_2, *J* = 6.8); 2.54 (br.s, 1 H, OH); 4.47 (d, 1 H, CHO, *J* = 5.4); 5.82 (s, 1 H, H(2)); 7.31 (d, 1 H, H(8), *J* = 7.6); 7.46 (d, 1 H, H(7), *J* = 7.6); 7.00 (br.s, 2 H, NH_2)	1620, 1645 (C=O); 2220 (C≡C); 3350, 3490 (NH_2); 3600 (OH)
4e	126—127	71.44 71.62	6.06 6.01	10.51 10.44	$C_{16}H_{16}N_2O_2$	1.25 (t, 6 H, 2 Me, *J* = 6.9); 3.50 (q, 4 H, 2 NCH_2, *J* = 6.9); 3.55 (s, 1 H, C≡CH); 5.82 (s, 1 H, H(2)); 7.34 (d, 1 H, H(8), *J* = 8.2); 7.58 (d, 1 H, H(7), *J* = 8.2); 7.10 (br.s, 2 H, NH_2)	1620, 1650 (C=O); 2100, 3310 (C≡CH); 3350, 3490 (NH_2)
7	143—144	74.12 74.18	5.26 5.41	7.66 7.52	$C_{23}H_{20}N_2O_3$	1.30 (t, 6 H, 2 Me, *J* = 7.0); 3.52 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.85 (s, 1 H, H(2)); 7.41 (d, 1 H, H(8), *J* = 7.8); 7.78 (d, 1 H, H(7), *J* = 7.8); 7.45—7.60 (m, 2 H, *m*-H arom.); 7.60—7.70 (m, 1 H, *p*-H arom.); 8.19 (dd, 2 H, *o*-H arom., $J_{o,m}$ = 8.5, $J_{o,p}$ = 1.5)	1650 (C=O); 2190 (C≡C); 3360, 3495 (NH_2)
8	71—72	70.93 70.98	6.52 6.55	8.41 8.28	$C_{20}H_{22}N_2O_3$	1.27 (d, 6 H, CMe_2, *J* = 6.9); 1.28 (t, 6 H, 2 NCMe, *J* = 7.0); 2.77 (sept, 1 H, CH, *J* = 6.9); 3.51 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.84 (s, 1 H, H(2)); 7.37 (d, 1 H, H(8), *J* = 7.8); 7.66 (d, 1 H, H(7), *J* = 7.8)	1660 (C=O); 2190 (C≡C); 3350, 3470 (NH_2)
9	209—210	71.82 71.92	3.31 3.49	4.21 4.41	$C_{23}H_{20}N_2O_3$	6.30 (s, 1 H, H(2)); 7.48 (d, 1 H, H(8), *J* = 7.7); 7.86 (d, 1 H, H(7), *J* = 7.7); 7.50—7.75 (m, 4 H, OH, *m*-H arom., *p*-H arom.); 8.10—8.25 (m, 2 H, *o*-H arom.)	1610, 1640 (C=O); 2200 (C≡C); 3370, 3490 (NH_2)

The chloroform solution was washed with water (5×70 mL), and $CHCl_3$ was removed *in vacuo* by replacing it with toluene during distillation. The toluene solution (~15 mL) was diluted with hexane (45 mL) and allowed to stand at 0 °C for ~12 h. The precipitate that formed was filtered off. The yield of compound **2** was 2.80 g (87.5%), decomposes at >100 °C (toluene—hexane). Found (%): C, 45.65; H, 4.22; I, 34.20. $C_{14}H_{15}IN_2O_2$. Calculated (%): C, 45.42; H, 4.08; I, 34.28. ^{1}H NMR, δ: 1.25 (t, 6 H, 2 Me, *J* = 6.9 Hz); 3.49 (q, 4 H, 2 NCH_2, *J* = 6.9 Hz); 5.85 (s, 1 H, H(2)); 7.10 (br.s, 2 H, NH_2); 7.11 and 7.93 (both d, 1 H each, H(7), H(8), *J* = 7.6 Hz).

5-Amino-3-diethylamino-6-phenylethynyl-1,4-naphthoquinone (4a). Phenylacetylene (**3a**) (0.17 g, 0.18 mL, 1.7 mmol), $Pd(PPh_3)_2Cl_2$ (18 mg), CuI (18 mg), and a solution of Na_2CO_3 (0.23 g, 2.3 mmol) in water (8 mL), which was preheated to ~80 °C, were successively added to a solution of compound **2** (0.40 g, 1.1 mmol) in dioxane (17 mL) under argon at 70 °C. The reaction mixture was stirred at 87 °C for 15 min, cooled, poured into water (400 mL), and extracted with toluene. The toluene solution was concentrated *in vacuo* and applied on a small Al_2O_3 layer (30×50 mm). Product **4a** was eluted with toluene. The yield was 0.30 g (81.1%) (see Table 1).

Table 2. Selected physicochemical properties and spectroscopic characteristics of substituted benz[*g*]indole-6,9-diones **5a—c** and benzo[*h*]quinoline-7,10-diones **12a—c** and **13a,b**

Compound	M.p./°C (toluene—hexane)	Found / Calculated (%)			Molecular formula	^{1}H NMR, δ (*J*/Hz)	IR, ν/cm^{-1}	UV, λ/nm (ε)
		C	H	N				
5a	136—137	76.51 76.72	5.69 5.85	7.95 8.13	$C_{22}H_{20}N_2O_2$	1.31 (t, 6 H, 2 Me, *J* = 7.0); 3.58 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.82 (s, 1 H, H(7)); 6.84 (d, 1 H, H(3), *J* = 2.3); 7.30—7.60 (m, 3 H, *m*-H arom., *p*-H arom.); 7.70—7.95 (m, 4 H, *o*-H arom., H(4), H(5)); 10.5 (br.s, 1 H, NH)	1620, 1670, (C=O); 3460 (NH)	285 (2.31), 406 (1.12)
5b	93—94	71.55 71.62	5.87 6.01	10.62 10.44	$C_{16}H_{16}N_2O_2$	1.29 (t, 6 H, 2 Me, *J* = 7.0); 3.57 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.82 (s, 1 H, H(7)); 6.59 (m, 1 H, H(3), $J_{H(3),H(2)}$ = 3.1, $J_{H(3),H(1)}$ = 1.8); 7.41 (m, 1 H, H(2), $J_{H(2),H(3)}$ = 3.1, $J_{H(2),H(1)}$ = 2.2); 7.81 (d, 1 H, H(4) (H(5)), *J* = 8.1); 7.88 (d, 1 H, H(5) (H(4)), *J* = 8.1); 10.25 (br.s, 1 H, NH)	1620, 1670 (C=O); 3470 (NH)	277 (1.53), 394 (0.84)
5c	108—109	74.06 74.00	6.41 6.54	8.83 9.08	$C_{19}H_{20}N_2O_2$	1.29 (t, 6 H, 2 NCMe, *J* = 7.0); 2.15 (s, 3 H, C=CMe); 3.56 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.23, 5.54 (both s, 1 H each, 2 =CH); 5.81 (s, 1 H, H(7)); 6.54 (d, 1 H, H(3), *J* = 2.0); 7.72—7.81 (m, 2 H, H(4), H(5)); 10.27 (br.s, 1 H, NH)	1620, 1660 (C=O); 3470 (NH)	278 (2.23), 402 (1.11)
12a	107.5—108	76.32 76.51	6.52 6.65	9.31 9.56	$C_{28}H_{29}N_3O_2$	1.36 (t, 6 H, 2 Me, *J* = 7.0); 1.80—2.00 (m, 6 H, CH_2—CH_2—CH_2); 3.10—3.50 (m, 4 H, CH_2—N—CH_2); 3.56 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.76, 7.39 (both s, 1 H each, H(8), H(3)); 7.30—7.60 (m, 3 H, *m*-H arom., *p*-H arom.); 8.08 (d, 1 H, H(5) (H(6)), *J* = 8.6); 8.20—8.45 (m, 3 H, *o*-H arom., H(6) (H(5)))	1620, 1690 (C=O)	276 (1.85), 401 (0.42)
12b	133—134	73.37 73.45	6.22 6.16	9.28 9.52	$C_{27}H_{27}N_3O_3$	1.37 (t, 6 H, 2 Me, *J* = 7.0); 3.27 (br.t, 4 H, CH_2—N—CH_2, *J* = 4.4); 3.57 (q, 4 H, 2 NCH_2, *J* = 7.0); 4.02 (br.t, 4 H, CH_2—O—CH_2, *J* = 4.4); 5.74, 7.40 (both s, 1 H each, H(8), H(3)); 7.45—7.60 (m, 3 H, *m*-H arom., *p*-H arom.); 8.11 (d, 1 H, H(5) (H(6)), *J* = 8.6); 8.25 (d, 1 H, H(6) (H(5)), *J* = 8.6); 8.15—8.30 (m, 2 H, *o*-H arom.)	1630, 1690 (C=O)	277 (2.65), 390 (0.61)
12c	83—84	75.60 75.85	6.75 6.84	9.71 9.83	$C_{27}H_{29}N_3O_2$	1.18, 1.37 (both t, 6 H each, 2 C(3)NCMe, 2 C(9)NCMe, *J* = 7.0); 3.42, 3.57 (both q, 4 H each, 2 C(3)NCH_2, 2 C(9)NCH_2, *J* = 7.0); 5.76, 7.40 (both s, 1 H each, H(8), H(3)); 7.40—7.60 (m, 3 H, *m*-H arom., *p*-H arom.); 8.06 (d, 1 H, H(5) (H(6)), *J* = 8.6); 8.10—8.35 (m, 3 H, H(6) (H(5)), *o*-H arom.)	1620, 1680 (C=O)	278 (3.22), 405 (0.74)
13a	92—93	73.78 74.04	7.71 7.71	10.21 10.36	$C_{25}H_{31}N_3O_2$	1.20—1.45 (m, 12 H, 2 NCMe, CMe_2); 2.95—3.25 (m, 5 H, CH=, CH_2NCH_2); 1.70—1.95 (m, 6 H, CH_2—CH_2—CH_2); 3.53 (q, 4 H, 2 NCH_2, *J* = 7.0); 5.71, 6.78 (both s, 1 H each, H(8), H(3)); 8.01 (d, 1 H, H(5) (H(6)), *J* = 8.6); 8.17 (d, 1 H, H(6) (H(5)), *J* = 8.6)	1620, 1680 (C=O)	285 (3.70), 391 (1.16)
13b	110—111	70.58 70.74	7.09 7.17	10.52 10.31	$C_{24}H_{29}N_3O_3$	1.34 (t, 6 H, 2 NCMe, *J* = 7.1); 1.36 (d, 6 H, CMe_2, *J* = 6.9); 2.95—3.30 (m, 5 H, CH=, CH_2—N—CH_2); 3.53 (q, 4 H, 2 NCH_2, *J* = 4.5); 3.98 (br.t, 4 H, CH_2—O—CH_2, *J* = 4.5); 5.72, 6.81 (both s, 1 H each, H(8), H(3)); 8.05 (d, 1 H, H(5) (H(6)), *J* = 8.6); 8.17 (d, 1 H, H(6) (H(5)), *J* = 8.6)	1620, 1680 (C=O)	289 (1.81), 384 (0.55)

5-Amino-3-diethylamino-6-(3-hydroxy-3-methylbutynyl)-1,4-naphthoquinone (4b). The reaction of iodide **2** (2.20 g, 6.0 mmol) with 3-methylbut-1-yn-3-ol (**3b**) (0.90 g, 0.7 mL, 10.7 mmol) was carried out analogously to the synthesis of compound **4a**. The reaction time was 7 min. The reaction mixture was diluted with water and extracted with $CHCl_3$. The chloroform solution was filtered through an Al_2O_3 layer (30×50 mm). The yield of acetylenic alcohol **4b** was 1.70 g (87.0%) (see Table 1).

5-Amino-3-diethylamino-6-(3-hydroxy-3-phenylpropynyl)-1,4-naphthoquinone (4c). Compound **4c** was prepared analogously to alcohol **4b** from iodide **2** (0.39 g, 1.0 mmol) and 3-phenylprop-1-yn-3-ol (**3c**) (0.22 g, 0.22 mL, 1.7 mmol). The reaction time was 20 min. The yield of compound **4c** was 0.37 g (94.9%) (see Table 1).

5-Amino-3-diethylamino-6-(3-hydroxy-4-methylpentynyl)-1,4-naphthoquinone (4d). Compound **4d** was prepared analogously to alcohols **4b,c** from iodide **2** (0.54 g, 1.5 mmol) and 4-methylpent-1-yn-3-ol (**3d**) (0.33 g, 3.4 mmol). The condensation time was 10 min. The yield of alcohol **4d** was 0.36 g (79.5%) (see Table 1).

5-Amino-3-diethylamino-6-ethynyl-1,4-naphthoquinone (4e). A calcined KOH powder (1.00 g, 17.8 mmol) was added with stirring to a solution of compound **4b** (2.00 g, 6.1 mmol) in toluene (210 mL) at 70 °C. The reaction mixture was heated to 110 °C, stirred for 20 min, cooled, and filtered through a small Al_2O_3 layer. The solvent was removed *in vacuo*. Ethynylnaphthoquinone **4e** was isolated in a yield of 1.60 g (95%) (see Table 1).

8-Diethylamino-2-phenylbenz[*g*]indole-6,9-dione (5a). A mixture of compound **4a** (0.35 g, 1.0 mmol) in DMF (20 mL) was heated in the presence of cuprous phenylacetylide (0.08 g, 0.5 mmol) and CuCl (0.05 g, 0.5 mmol) under argon at 155 °C for 4.5 h, poured into water (400 mL), and extracted with toluene. The solvent was removed *in vacuo*. The yield of benzindoledione **5a** was 0.29 g (82.2%) (see Table 2).

8-Diethylaminobenz[*g*]indole-6,9-dione (5b). A mixture of compound **4e** (0.10 g, 0.4 mmol) and piperidine (4 mL) was heated at 70 °C for 3.5 h. Excess piperidine was distilled off *in vacuo*. The residue was dissolved in $CHCl_3$, applied onto SiO_2 (45×140 mm), and allowed to stand for 2 days. Benzindoledione **5b** was washed with $CHCl_3$ and a $CHCl_3$—EtOH mixture. The yield was 0.08 g (80%) (see Table 2).

8-Diethylamino-2-isopropenylbenz[*g*]indole-6,9-dione (5c). A mixture of acetylenic alcohol **4b** (0.60 g, 1.8 mmol) was subjected to cyclization in the presence of cuprous phenylacetylide (0.15 g, 0.9 mmol) and CuCl (0.09 g, 0.9 mmol) in DMF (35 mL) analogously to heterocyclization of compound **4a** in indole **5a**. The reaction time was 5 h. Chromatography on SiO_2 (toluene and a toluene—acetone mixture as the eluents) afforded isopropenylbenzindole **5c** in a yield of 0.31 g (55.4%) (see Table 2).

5-Amino-6-benzoylethynyl-3-diethylamino-1,4-naphthoquinone (7). ***A.*** The Collins reagent[24] (1.90 g, 7.3 mmol) was added portionwise with stirring to a cold solution of compound **4c** (0.54 g, 1.4 mmol) in dry freshly distilled CH_2Cl_2 (75 mL) for 10 min, so that the temperature of the reaction mixture was maintained no higher than 10 °C. The reaction mixture was stirred at 20 °C for 1 h, diluted with $CHCl_3$ (100 mL), and poured into a solution of $NaHCO_3$ (3.00 g, 35.7 mmol) in water (70 mL). The organic layer was separated, washed with water (3×100 mL), and dried with $MgSO_4$. The solvent was removed *in vacuo*. Ketone **7** was obtained in a yield of 0.50 g (94.3%) (see Table 1).

B. A mixture of iodide **2** (1.10 g, 2.8 mmol) and cuprous benzoylacetylide (0.80 g, 4.3 mmol) in DMF (30 mL) was stirred at 140 °C under argon for 1 h, cooled, diluted with benzene (200 mL), and repeatedly washed with water. After removal of the solvent *in vacuo*, the residue was chromatographed on SiO_2 (benzene and $CHCl_3$ as the eluents). Ketone **7** was crystallized from hexane. The yield of 0.70 g (66%).

C. A mixture of benzoyl chloride (0.52 g, 0.42 mL, 4.0 mmol) and Et_3N (0.60 g, 0.84 mL, 6.0 mmol) in benzene (8 mL) was stirred under argon (2—3 min). Then a solution of ethynylnaphthoquinone **4e** (0.54 g, 2.0 mmol) in benzene (10 mL) was added, the reaction mixture was warmed to 60 °C, and $Pd(PPh_3)_2Cl_2$ (30 mg) was added. Then the mixture was heated to 80 °C and stirred for 5 min. Chromatography on Al_2O_3 (30×80 mm) in benzene afforded ketone **7** (0.30 g, 42%).

5-Amino-3-diethylamino-6-(4-methyl-3-oxopentynyl)-1,4-naphthoquinone (8). Alcohol **4d** (0.80 g, 2.3 mmol) was oxidized with the Collins reagent (7.50 g, 29.0 mmol) in CH_2Cl_2 (150 mL) under the conditions of the synthesis of ketone **7**. The yield of ketone **8** was 0.60 g (78%) (see Table 1).

5-Amino-6-benzoylethynyl-3-hydroxy-1,4-naphthoquinone (9). A solution of ketone **7** (0.30 g, 0.8 mmol) and $HgSO_4$ (60 mg) in dioxane (20 mL) acidified with 45% H_2SO_4 (0.3 mL) was stirred at 85 °C for 4 h. Then the reaction mixture was cooled, poured into water (300 mL), and extracted with $CHCl_3$. The organic layer was washed with water to neutral pH and dried with $MgSO_4$. The solvent was removed *in vacuo* and the residue was triturated with hexane. Hydroxynaphthoquinone **9** was obtained in a yield of 0.23 g (76.7%) (see Table 1).

9-Diethylamino-4-piperidino-2-phenylbenzo[*h*]quinoline-7,10-dione (12a). A solution of ketone **7** (0.30 g, 0.8 mmol) in piperidine (12 mL) was stirred at 20 °C for 45 min. After completion of the reaction, the mixture was poured into water (300 mL) and extracted with benzene 100 mL. The product was isolated from the benzene extract with 12% HCl (2×45 mL). A hydrochloric acid solution was gradually alkalized with a 20% aqueous KOH solution (260 mL) and extracted with benzene (100 mL) as the mixture became neutral. After separation, the aqueous alkaline layer was additionally extracted with benzene (3×50 mL). The combined benzene extracts were washed with water to neutral pH and dried with $MgSO_4$. The solvent was removed *in vacuo*. Compound **12a** was obtained in a yield of 0.24 g (68.6%) (see Table 2).

9-Diethylamino-4-morpholino-2-phenylbenzo[*h*]quinoline-7,10-dione (12b). Compound **12b** was prepared analogously from ketone **7** (0.48 g, 1.3 mmol) and morpholine (13 mL) at 20 °C (2 h) in a yield of 0.52 g (91%) (see Table 2).

4,9-Bis(diethylamino)-2-phenylbenzo[*h*]quinoline-7,10-dione (12c). Compound **12c** was prepared analogously from ketone **7** (0.49 g, 1.3 mmol) and Et_2NH (27 mL) at 20 °C (1 h) in a yield of 0.30 g (55.6%) (see Table 2).

9-Diethylamino-2-isopropyl-4-piperidinobenzo[*h*]quinoline-7,10-dione (13a). Compound **13a** was prepared analogously from acetylenic ketone **8** (0.28 g, 0.8 mmol) and piperidine 18 mL at 20 °C (70 min). The yield of benzoquinolinedione **13a** was 0.17 g (51.3%) (see Table 2).

9-Diethylamino-2-isopropyl-4-morpholinobenzo[*h*]quinoline-7,10-dione (13b). Compound **13b** was prepared analogously from ketone **8** (0.30 g, 0.9 mmol) and morpholine (9 mL) at 20 °C

(105 min). The yield of benzoquinolinedione **13b** was 0.18 g (50%) (see Table 2).

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Review

Anti-*Trypanosoma cruzi* Compounds: Our Contribution for the Evaluation and Insights on the Mode of Action of Naphthoquinones and Derivatives

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A doença de Chagas causada pelo *Trypanosoma cruzi* afeta cerca de oito milhões de pessoas em países em desenvolvimento, sendo classificada como uma doença tropical negligenciada pela Organização Mundial da Saúde. A quimioterapia disponível para esta doença é baseada em dois nitro-heterocíclicos, nifurtimox e benznidazol, ambos com graves efeitos colaterais e eficácia variável, e assim novos medicamentos visando um tratamento mais eficiente são necessários com urgência. Nos últimos 20 anos, temos desenvolvido em colaboração com grupos focados em química medicinal, um programa de quimioterapia experimental da doença de Chagas, investigando a eficácia, seletividade, toxicidade, alvos celulares e mecanismos de ação de diferentes classes de compostos sobre *T. cruzi*. Neste artigo, apresentamos uma visão geral desses estudos, enfocando protótipos naftoquinoidais e derivados, examinando a sua síntese, a atividade e mecanismo de ação, o que foi realizado e o que precisa ser abordado, avaliando e discutindo possíveis melhorias. Esta mini-revisão discute nosso esforço continuado visando a caracterização biológica e a síntese de compostos naftoquinoidais, auxiliando no desenvolvimento de um novo arsenal de drogas candidatas com eficácia contra o *T. cruzi*.

Chagas disease is caused by the parasite *Trypanosoma cruzi* and affects approximately eight million individuals in the developing world; it is also classified as a neglected tropical disease by the World Health Organization. The available therapy for this disease is based on two nitroheterocycles, nifurtimox and benznidazole, both of which exhibit severe side effects and variable efficacy; therefore, new drugs and better treatment schedules are urgently needed. For the past 20 years, we have been collaborating with groups focused on medicinal chemistry to produce experimental therapies for Chagas disease by investigating the efficacy, selectivity, toxicity, cellular targets and mechanisms of action of different classes of compounds against *T. cruzi*. In this report, we present an overview of these studies, focusing on naphthoquinonoid prototypes and discuss their synthesis, activity and mechanisms of action. Furthermore, we summarise the research that has been performed to date and suggest future research directions while assessing and discussing potential improvements. This mini-review discusses our continued efforts toward the biological characterisation and synthesis of naphthoquinoidal compounds, aiming to contribute to the development of a new arsenal of candidate drugs that exhibit effective anti-*T. cruzi* activity

Keywords: naphthoquinones, β-lapachone, *Trypanosoma cruzi*, Chagas disease, chemotherapy

1. Introduction

Chagas disease (CD) is caused by the intracellular obligatory parasite *Trypanosoma cruzi* and was first described more than one hundred years ago, in 1909, by Carlos Chagas.[1] This disease has high morbidity and mortality rates, affects approximately eight million individuals in the developing world and displays a limited response to therapy; it has also been classified as a neglected tropical disease by the World Health Organization (WHO).[2,3] Chagas disease can be transmitted through the faeces of sucking Triatominae insects, blood transfusions, organ transplantation, oral

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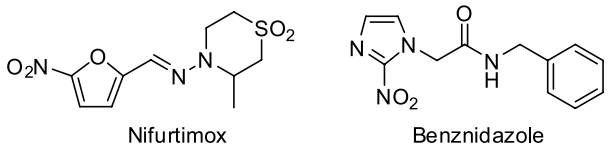
contamination, through laboratory accidents and congenital routes. *T. cruzi* is a hemoflagellate protozoan (family Trypanosomatidae, order Kinetoplastida)[4] that exhibits a complex life cycle involving distinct morphological stages during its passage through vertebrate and invertebrate hosts. Briefly, after ingestion of bloodstream trypomastigotes by insect vectors, the parasites are converted to epimastigote forms, which proliferate and subsequently differentiate into metacyclic forms within the posterior intestine of the triatomine. These infective parasite forms are released in the faeces of the triatomine and can invade new vertebrate cells. The parasites then undergo another round of differentiation into intracellular amastigote forms, which proliferate and subsequently transform back into trypomastigotes, the form that disseminates the infection.

Although vector and transfusion transmissions have sharply declined over the past 20 years due to the Southern Cone countries policy, several challenges still need to be overcome including those related to sustainable disease control through the adoption of public policies in the endemic areas.[5,6] In addition, despite effective efforts to control vector and blood transmission, Chagas disease still presents many challenges including the following: (*i*) its peculiar epidemiology is characterised by a variety of risk factors (many potential vectors and reservoirs, different forms of transmission and diverse parasite isolates present in domiciliar, peridomiciliar and sylvatic environments); and importantly, (*ii*) the lack of prophylactic therapies and effective therapeutic treatments.[7,8] Current major concerns include disease transmission by the ingestion of contaminated food or liquids and the disease's emergence in nonendemic areas such as North America and Europe, a phenomenon which is likely due to the immigration of infected individuals.[9,10] This disease is also recognised as an opportunistic infection in HIV-infected individuals.[11] Outbreaks of acute Chagas disease associated with the ingestion of contaminated food and drink have been reported in South America,[12,13] and are associated with a high mortality rate mainly due to myocarditis.

Chagas disease is characterised by two clinical phases. The acute phase appears shortly after infection, and in some cases the individual may not even realise he/she is infected. Symptoms range from flu-like symptoms to intense myocarditis (in approximately 10% of infected people). If left untreated, symptomatic chronic disease develops in about one third of the individuals after a long latent period (10-30 years) that is known as the indeterminate form. The main clinical manifestations of Chagas disease include digestive and/or cardiac alterations, although disorders of the central and peripheral nervous system may also occur.[14,15] In the chronic digestive form of the disease, the clinical manifestations are caused by dysperistalsis of the oesophagus and colon, which are due to the destruction of the myenteric plexus and leads to mega syndromes.[16] The chronic cardiac form of the disease is the most significant clinical manifestation, and consequences include dilated cardiomyopathy, congestive heart failure, arrhythmias, cardioembolism and stroke.[17] The pathogenesis of Chagas disease is the result of a sustained inflammatory process due to an anti-parasitic and/or anti-self-immune response, which is associated with low-grade persistent parasite presence.[18-22] Growing evidence shows that parasite persistence within the target organs associated with an unregulated host immune response are involved in disease progression and clinical outcomes.[19,23] Control of *T. cruzi* infection depends on both the innate and acquired immune responses which are triggered during early infection and are critical for host survival. These responses involve macrophages, natural killer cells, T and B lymphocytes and the production of pro-inflammatory cytokines.[24]

The available therapy for Chagas disease is based on two nitroheterocyclic agents that were developed over five decades ago (Figure 1).



Nifurtimox Benznidazole

Figure 1. Chemical structures of nifurtimox and benznidazole.

Nifurtimox (Nif, 3-methyl-4-(5'-nitrofurfurylidene-amine)tetrahydro-4*H*-1,4-tiazine-1,1-dioxide) is a nitrofuran that was developed by Bayer in 1967 and marketed as Lampit®. It acts by reducing the nitro group to generate nitro-anions that subsequently react with molecular oxygen to produce toxic superoxide and peroxide radicals. Today, Nif is produced by Bayer HealthCare at the Corporacion Bonima in El Salvador. Benznidazole (Bz, *N*-benzyl-2-nitroimidazole acetamide) is a nitroimidazole that was developed by Roche in 1972 and was formerly marketed as Rochagan® or Radanil®; it is currently produced by the Laboratório Farmacêutico do Estado de Pernambuco, Brazil (www.pe.gov.br/orgaos/lafepe-laboratorio-farmaceutico-de-pernambuco/). This drug appears to act differently, as it produces metabolites that react with macromolecules such as DNA, RNA, proteins, and possibly lipids. In both cases, the antiparasitic activity of the drug is intimately linked with their inherent toxicity. Both drugs are effective against acute infections, but they show poor activity during the late chronic phase.[16] Due to their severe side effects and limited efficacy against

different parasitic isolates,[25] these drugs are hardly the best treatment options to offer patients. One of the major drawbacks of Nif is its high incidence of side effects, which is observed in up to 40% of patients and includes nausea, vomiting, abdominal pain, weight loss and severe anorexia. Furthermore, adverse neurological effects such as restlessness, paresthesia, twitching, insomnia and seizures have also been observed.[21] In comparison to Nif, Bz has the advantage of a lower incidence of side effects; however, its side effects include hypersensitivity (dermatitis, generalised oedema, ganglionic infarction and joint and muscle pains), bone marrow depletion and peripheral polyneuropathy.[26] Because of the challenges regarding the efficacy *vs*. the toxicity of both nitro-heterocyclic compounds, the current recommendations for using either drug to treat Chagas disease suggest that all acute cases, including reactivations due to immunosuppression, recent chronic cases (including children up to 12 years of age), and indeterminate or benign chronic forms should be treated. In addition, cases should be treated at the discretion of the attending physician. In contrast, the contra-indications for specific treatment are pregnancy, liver and kidney failure, neurological diseases unrelated to CD, advanced CD with grade III or IV cardiopathy (Pan American Health Organization, PAHO)/(WHO), or other pathologies that may be worsened by treatment.[26] Between 12 and 18% of patients who undergo treatment have to suspend their therapy prematurely because of side effects.[27] Overall, the 2010 Latin American Guidelines for Chagas cardiomyopathy indicate that unrestricted treatment for patients with chronic Chagas disease should not be regarded as standard therapy.[28]

Several new compounds are currently under preclinical development, and different approaches have been used to identify new drug leads including *in vitro* parasite phenotype screens and target-based drug discovery.[29] Although many attempts have been made to treat the disease since its identification in 1912, only allopurinol and some antifungals have been used in clinical trials since the introduction of Nif and Bz.[25,30] In 2009, the Drugs for Neglected Diseases initiative (DND*i*) and its partners launched the Chagas disease Clinical Research Platform (http://www.dndi.org/strenghtening-capacity/chagas-platform/publications.html), which aims to promote technical discussions, develop a critical mass of expertise, strengthen institutional research capacities, and link investigators through a collaborative network. As a result, three phase II clinical trials began in 2011 to investigate the potential uses of posaconazole (a structural analogue of itraconazole) (SCH 56592; Schering-Plough Research Institute, SPRI) and of a prodrug of ravuconazole (E1224; Eisai) (Figure 2).

Both drugs are triazole derivatives that inhibit fungal and protozoan cytochrome P-450-dependent enzyme CYP51 (C14α-lanosterol demethylase) (TcCYP51).[31-33] Two clinical studies were performed with posaconazole: STOP-CHAGAS (in Argentina, Colombia, Mexico and Venezuela, funded by Merck) with results expected by 2014 and CHAGASAZOL (in Spain at University Hospital Vall d'Hebron Research Institute in Barcelona), which was completed in March 2013 (results were posted at http://clinicaltrials.gov/show/NCT01162967, accessed in July, 2014). Another study investigated the use of E1224 (DND*i*/Eisai Pharmaceuticals) and was developed in Bolivia. It involved a total of 231 patients, and the drug exhibited a good safety profile and was effective at clearing the parasite; however, it had little to no sustained efficacy one year after treatment. The key disadvantages of novel azole derivatives (i.e., posaconazole) are their complexity and manufacturing costs.[31]

Among the drugs identified in preclinical studies, several of them have yielded valuable results. For example, CYP51 inhibitors such as tipifarnib (an anti-cancer drug that inhibits the human protein farnesyltransferase)[32] and the fenarimol series show promise.[33] In addition, fexinidazole (a substituted 5-nitroimidazole that was rediscovered by the DND*i* and is currently in phase II/III clinical study for the treatment of human African trypanosomiasis),[34] diamidine analogues[35] and a series of oxaboroles (prototype AN4169) are promising new drugs for the treatment of *T. cruzi* infections.[36] Other drug targets under investigation include cysteine proteases because *T. cruzi* contains a cathepsin L-like enzyme (cruzipain) that is responsible for the majority of the proteolytic activity that occurs in all developmental forms. The vinyl

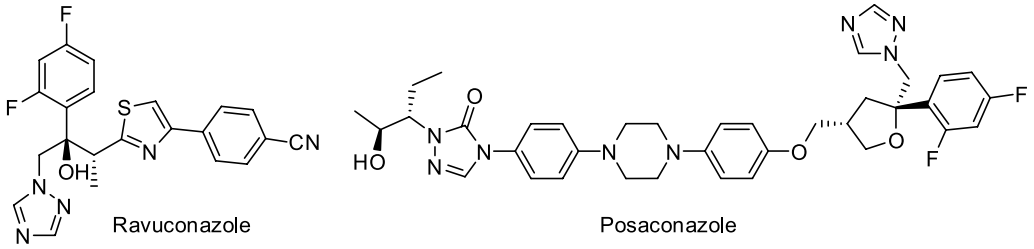


Figure 2. Chemical structures of posaconazole and ravuconazole.

sulfone K777 is an irreversible cruzipain inhibitor that has shown efficacy in chronic rodent models and is also under preclinical development.[29] Some of the most promising targets identified in *T. cruzi* include protein prenylation, hypoxanthine-guanine phosphoribosyltransferase, cysteine proteases,[29,37] and topoisomerases.[38] The utility of 14-demethylase inhibitors,[39,40] squalene synthase inhibitors,[41] farnesyl pyrophosphate synthase inhibitors,[42] farnesyl transferase inhibitors,[43,44] dihydrofolate reductase inhibitors[45] and natural products such as canthinones, quinolines, lignans, and naphthoquinones are also being explored.[46-48] New and established pharmacophores based on synthetic and natural product chemistry have been identified through improved screening technologies and have generated hits from libraries provided largely by the pharmaceutical industry and other entities.

Another approach aimed at the treatment of Chagas disease is the achievement of greater efficacy through the use of combinations of existing drugs that display different mechanisms of action. Combination therapy has been proven to be more effective than monotherapies for several infectious diseases and also minimises the risk of drug resistance. Several studies in animal models have examined the use of combinations of Bz and CYP51 inhibitors,[49-52] the arylimidamide DB766,[53] and allopurinol,[54,55] and the results were encouraging. Coura[26] proposed the use of combinations of [Nif + Bz], [Nif or Bz + allopurinol] and [Nif or Bz + ketoconazole, fluconazole or itraconazole] in specified treatment schemes that were adapted according to the side effects observed.

Based on current knowledge of parasite and host biological characteristics, a desired drug candidate for Chagas disease would include the following attributes: (*i*) high activity against the evolving forms of the parasite present in the mammalian hosts and different reservoirs of the parasite, (*ii*) efficacy against both acute and chronic infections, (*iii*) oral administration of only a few doses, (*iv*) low toxicity and an improved safety profile (including children and women of reproductive age), (*v*) low cost and high stability suitable for a long shelf life in tropical temperatures, and (*vi*) high levels of tissue accumulation and long terminal half-lives.[55]

Over the past 20 years, our group has been working on experimental chemotherapy for Chagas disease in collaboration with research groups focused on medicinal chemistry. We have been investigating the efficacy, selectivity, toxicity, cellular targets and mechanisms of action of different classes of compounds on *T. cruzi*. In this report, we present an overview of these studies, focusing on the development of novel naphthoquinonoid prototypes for the clinical treatment of Chagas disease. We also describe their synthesis, activity and mechanisms of action. Furthermore, we summarise the current state of research in the field and suggest future directions while assessing and discussing potential improvements. This mini-review discusses our continued efforts toward the biological characterisation and synthesis of naphthoquinoidal compounds, aiming to contribute in the development of a new arsenal of candidate drugs that exhibit effective anti-*T. cruzi* activity.

2. Quinoidal Compounds and Derivatives

Quinoidal compounds can be found in various plant families or as synthetic substances.[56-59] The structural components of these compounds are the focus of many studies attempting to determine their activity against several parasites such as *Leishmania*,[60] *T. cruzi*[61] and *Plasmodium falciparum*.[62] Quinones participate in multiple biological oxidative processes due to their structural properties and their capacity to generate reactive oxygen species.[63,64]

The first report published in collaboration with Antonio V. Pinto's group from the Federal University of Rio de Janeiro in 1994 described a series of natural and synthetic drugs that exhibited activity against *T. cruzi*.[65] In this work, we evaluated 45 compounds for activity against bloodstream forms of *T. cruzi*. From there, a fruitful partnership began, and several molecules were synthesised and screened for activity against this parasite.

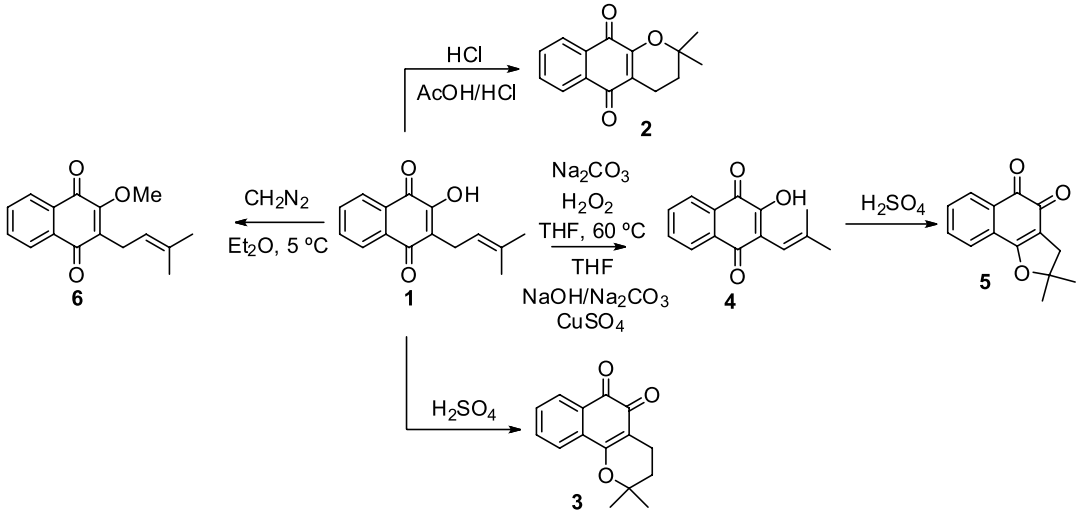
Following this initial study, we dedicated our efforts to the identification of new quinoidal molecules. Lapachol (**1**) is an important natural naphthoquinone; we used it and its derivatives to explore the chemical reactivity of the drug class, and several heterocycles were obtained with good yields (Schemes 1-3). Their effects on the bloodstream forms of *T. cruzi* were evaluated, and the results are shown in Table 1. Some compounds that exhibited initial activity were identified as potential candidates for further studies due to comparable activity with crystal violet, a substance indicated for the sterilisation of chagasic blood.[66] Unless otherwise stated, all of the screening assays presented in this review were performed using bloodstream trypomastigotes of the Y strain obtained from infected albino mice at the peak of parasitaemia. These trypomastigotes were isolated by differential centrifugation and resuspended (10^7 cells mL^{-1}) in Dulbecco's modified Eagle medium containing 10% mouse blood. This parasite suspension (100 μL) was added to the same volume of each previously prepared compound at twice the desired final concentrations in 96-well microplates and was incubated for 24 h at 4 °C. For experiments using epimastigotes (Y strain), the parasites were maintained axenically at 28 °C with

weekly transfers of liver infusion tryptose (LIT) medium and harvested during the exponential phase of growth (5-day-old culture forms). The assays were performed in 24-well microplates and were incubated up to 4 days at 28 °C in LIT medium. Cell counts were performed in a Neubauer chamber, and trypanocidal activity was expressed as an IC_{50} value corresponding to the concentration that lyses 50% of the parasites.

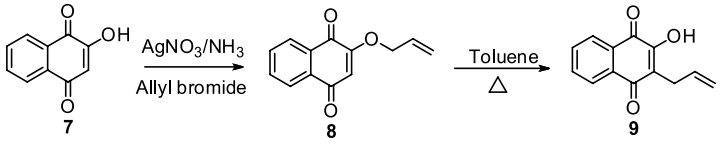
Meanwhile, we reported the synthesis and evaluation of naphthoxazoles containing both electron donating and withdrawing groups (Figure 3).[67,68] Heterocycles, as for instance, indole and 1,3-benzodioxole, as substituent groups were also evaluated. The compounds were easily obtained from the reaction of β-lapachone or nor-β-lapachone and aromatic aldehydes in the presence of an ammonium salt. In general, these structures exhibited efficient anti-*T. cruzi* activity and represented an excellent starting point for the synthesis of new prototypes.

Another class of structures prepared from the same reaction were the naphthoimidazole derivatives **27**-**39** (Figure 4). The trypanocidal activities of the naphthoxazoles **19**-**26** and naphthoimidazoles **27**-**39** are displayed in Table 2. From these substances, compounds **18** (IC_{50} = 37.0 ± 0.7 μM), **27** (IC_{50} = 15.4 ± 0.2 μM) and **39** (IC_{50} = 15.5 ± 2.9 μM) were selected for further studies of the trypanocidal mechanism of action.[69]

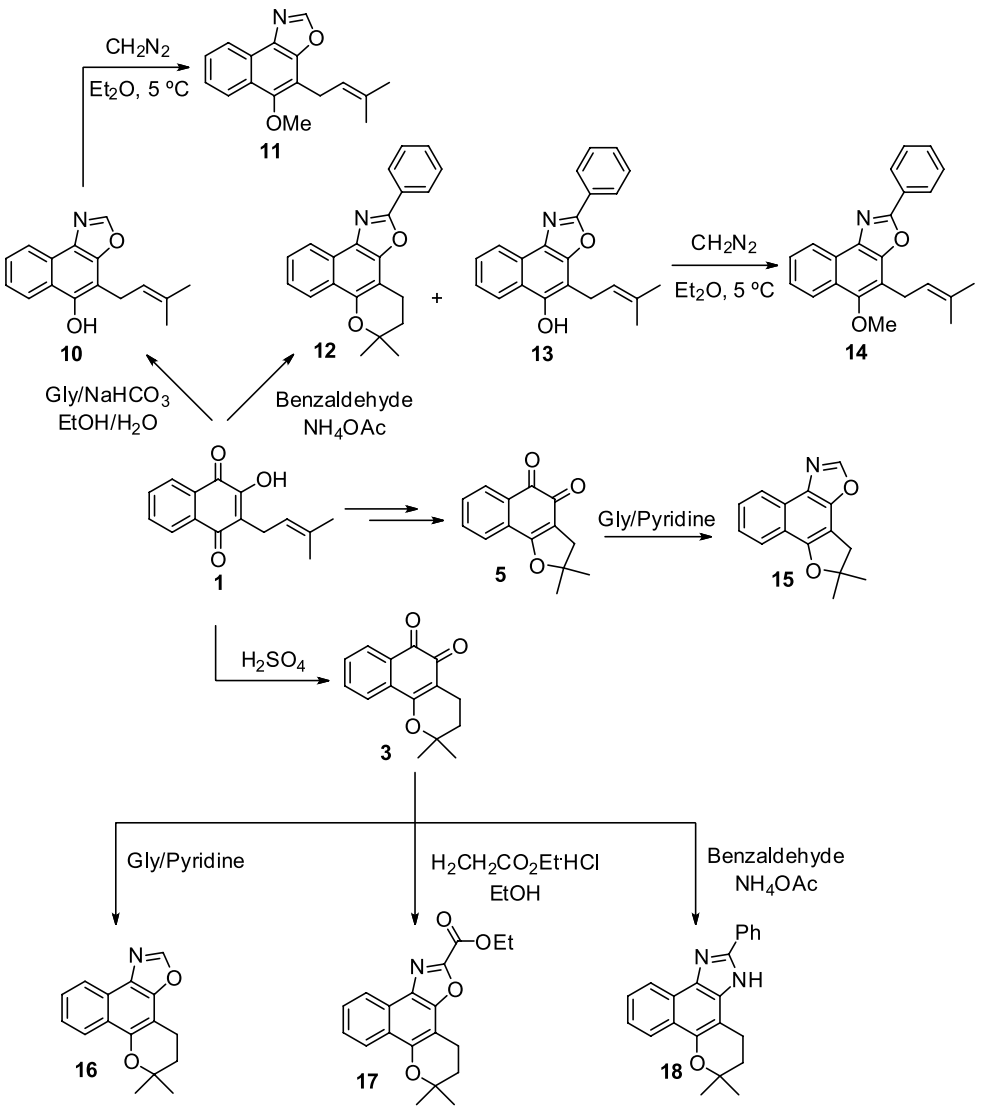
The naphthoimidazoles **18**, **27** and **39** were also effective against the proliferative forms of *T. cruzi* (intracellular amastigotes and epimastigotes), and the main ultrastructural targets identified were the mitochondrion and nuclear DNA.[70] Electron microscopy analyses revealed mitochondrial swelling, abnormal chromatin condensation, endoplasmic reticulum profiles surrounding organelles and autophagosome-like structures in treated parasites. We also observed reservosome disorganisation and trans-Golgi network cisternae disruption specifically in the epimastigote forms.[70,71] Interestingly, the pre-incubation of the parasites with the cysteine protease inhibitor E64 or calpain inhibitor I partially attenuated the trypanocidal effect of the naphthoimidazoles suggesting that the deactivation of cysteine proteases is involved in their mode of action.[70] Because the reservosome is a target in epimastigotes and is rich in cysteine proteases, disruption of this organelle could release proteases into the cytosol and initiate a proteolytic pathway, ultimately leading to parasite death. Alterations of mitochondrion, chromatin, and reservosomes and the detection of an autophagy process encouraged further studies regarding death pathways. The investigation of the apoptotic features demonstrated discrete phosphatidylserine exposure and strong DNA fragmentation by both electrophoresis and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) techniques.[70-72] Naphthoimidazoles are planar in structure and could possibly interact with the parasite's DNA to induce fragmentation, which is a decisive event during trypanocidal activity. In contrast,



Scheme 1. Synthetic route for the preparation of lapachol derivatives **1**-**6**.[66]



Scheme 2. Lawsone **7** and its derivatives **8** and **9**.[66]



Scheme 3. Synthetic route for the attainment of compounds **9**-**18**.[66]

the morphological evidence of autophagy induction after treatment with compounds **18**, **27** and **39** stimulated a more detailed evaluation of this pathway. Strong labelling of monodansylcadaverine (an autophagosome probe) together with ATG (autophagic-related genes) overexpression and total abolition of the compounds' effects by the well-known autophagic inhibitors wortmannin or 3-methyladenine in both treated epimastigotes and trypomastigotes supported the hypothesis that autophagy was involved in the naphthoimidazoles' mode of action.[72] However, further proteomic analysis is needed to identify *T. cruzi* molecules involved in the mechanism of action of compounds **18**, **27** and **39**. In 2010, the first assessment of the proteomic profile of naphthoimidazole-treated epimastigotes was performed. Multiple biochemical pathways were involved in their trypanocidal activity including redox metabolism, energy production, ergosterol biosynthesis, cytoskeleton assembly, protein metabolism and chaperone modulation. An imbalance among these fundamental pathways could lead to the loss of homeostasis and culminate in *T. cruzi* death.[73] Among the proteins modulated by the treatment, 26 proteins were downregulated, and only three proteins were overexpressed. Surprisingly, most of the modulated proteins were exclusive to each particular compound, indicating that differences in their modes of action existed (Figure 5).

Mitochondrial proteins were the most commonly modulated proteins, thus confirming the previous biochemical and ultrastructural evidence that described this organelle as the primary target of these compounds.[70,71,73] Tubulin was downregulated in parasites treated with compounds **18**, **27** and **39**. In trypanosomatids, different tubulin isoforms are present because each one is linked to the kinetics of microtubule assembly. Enzyme-linked immunosorbent assay (ELISA) data showed that the tyrosinated tubulin

Table 1. Effects of the original quinones and their naphthoxazole and naphthoimidazole derivatives on *T. cruzi*

Compound	IC_{50}, 24 h / μM[a]
1	410.8 ± 53.5
2	> 4800
3	391.5 ± 16.5
4	1280.6 ± 167.2
5	> 400
6	164.8 ± 30.5
7	> 2500
8	420.7 ± 71.2
9	330.7 ± 62.4
10	> 2500
11	49.5 ± 1.4
12	283.5 ± 25.0
13	171.9 ± 51.2
14	197.3 ± 25.8
15	> 2500
16	325.2 ± 21.3
17	> 4800
18	37.0 ± 0.7
Crystal violet	536.0 ± 3.0

[a]Mean ± standard deviation from three experiments performed in triplicate.

pool decreased after treatment. This protein isoform was associated with labile microtubules, suggesting that these compounds interfered with intracellular vesicle traffic and/or mitotic spindle formation. This hypothesis was also supported by the absence of ultra-structural damage in subpellicular and flagellar microtubules and the blockage of mitosis in treated epimastigotes.[70,71,73] Due to the results obtained about the activity and mechanism of action of **18**, **27** and **39** higher amounts of the compounds were synthesised and experiments are underway in our laboratory aiming the evaluation of nitroimidazoles in the treatment of experimentally *T. cruzi*-infected mice.[74]

To synthesise new heterocycles, Pinto and co-workers[67] developed a methodology to produce pyran derivatives of β-lapachone (**3**) through a reaction using active methylene reagents under basic conditions. The resulting cyclopentenones and chromenes were evaluated for anti-*T. cruzi* activity in addition to the other heterocyclic compounds shown in Figure 6. The results of the trypanocidal activity studies are shown in Table 3. Unfortunately, this class of compounds did not exhibit trypanocidal activity comparable to that of the naphthoimidazole derivatives, with the exception of compound **45**. Thus, these substances have not been the subject of subsequent studies.

In the same manner, we continued the search for trypanocidal heterocyclic compounds and obtained a phenazine derivative **50** (Figure 7) from β-lapachone (**3**), which was subsequently well characterised by crystallographic methods. This compound was almost twice as active as Bz, with an IC_{50} (24 h) of 61.3 ± 9.6 μM.[75] Despite its promising activity level, the yield for obtaining compound **50** from lapachone (**3**) was low (25% yield), which discouraged further studies. However, phenazines obtained from lapachones generally exhibited low levels of cytotoxicity,[76] and this phenazine represents an important prototype for the design of novel trypanocidal drugs.

Over the last few years, our group has focused on synthesising and measuring the trypanocidal activity of nor-β-lapachones substituted with heterocyclic rings. In general, a molecular hybridisation strategy was used to design the new compounds,[77] and the subject of our study was the combination of a quinoidal moiety with a 1,2,3-triazole group. The first synthetic route we developed followed the principles of medicinal chemistry and

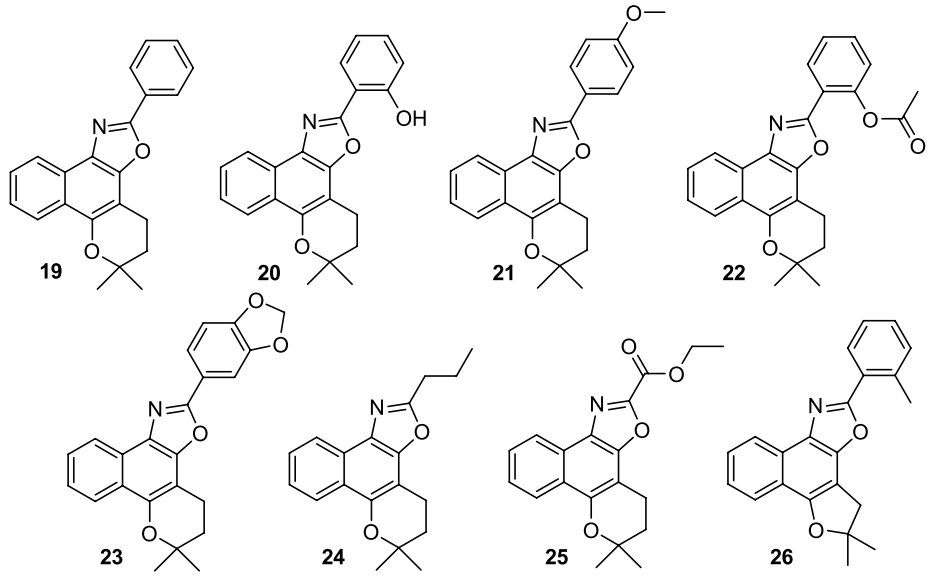


Figure 3. Naphthoxazoles **19**-**26** obtained from β-lapachone (**3**) and nor-β-lapachone (**5**).[67,68]

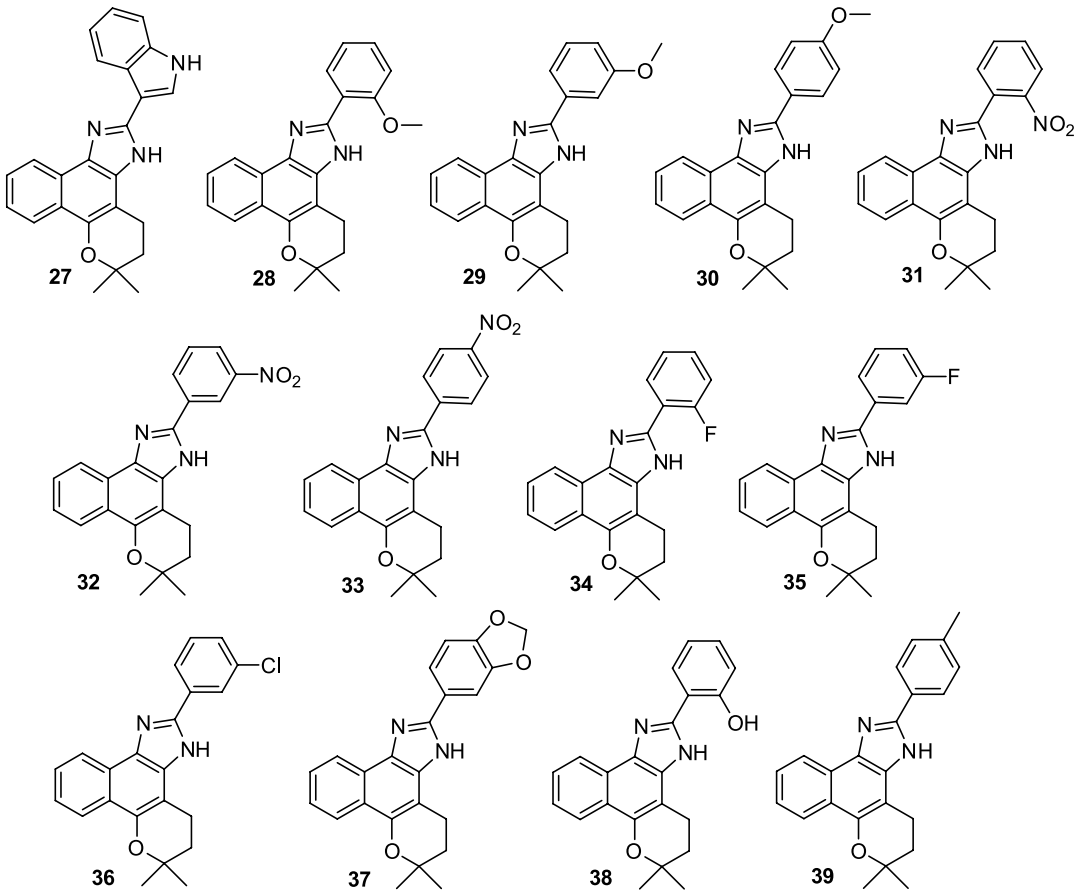


Figure 4. Naphthoimidazoles **27**-**39** obtained from β-lapachone (**3**).[67,68]

Table 2. Effects of naphthoxazoles and naphthoimidazoles on *T. cruzi*

Compound	IC_{50}, 24 h / μM[a]
19	283.5 ± 25.0
20	> 9600
21	3502.5 ± 305.3
22	1641.3 ± 147.0
23	269.5 ± 46.5
24	351.4 ± 12.4
25	> 4800
26	> 2500
27	15.4 ± 0.2
28	6444.6 ± 483.7
29	3057.8 ± 836.7
30	259.3 ± 40.4
31	1858.1 ± 366.7
32	579.3 ± 52.5
33	303.6 ± 12.2
34	243.3
35	372.0
36	1064.2
37	1850.5
38	4455.5 ± 465.8
39	15.5 + 2.9
Benznidazole	103.6 ± 0.6

[a]Mean ± standard deviation from three experiments performed in triplicate.

produced lapachone-based 1,2,3-triazoles with global yields higher than 50%. Using the Hooker oxidation method,[78] nor-lapachol (**4**) was prepared and used to obtain the key intermediate 3-azido nor-β-lapachone (**51**). Compound **51** was used to prepare the respective 1,2,3-triazole derivatives **52**-**61** by employing a 1,3-dipolar reaction catalysed by Cu(I), a type of reaction also known as "click chemistry" (Scheme 4).[79] The results of the trypanocidal activity studies are shown in Table 4.[80,81]

Overall, all compounds exhibited good trypanocidal activity, and several compounds were even more active than Bz. It was recently suggested in the Perspectives Section of the Journal of Medicinal Chemistry[82] that a triazolic naphthofuranquinone compound (**56**) represents an important trypanocidal prototype. Compound **56** was the most active with an IC_{50} (24 h) value of 17.3 ± 2.0 μM, and this substance was chosen for further studies of its mechanism of action.[83] This compound was also effective against the epimastigote and intracellular amastigote forms of *T. cruzi*, with IC_{50} (24 h) values below 25 μM. Scanning electron microscopy analyses revealed bizarre multiflagellar parasites in the treated group that also exhibited abnormal morphology during parasite division.

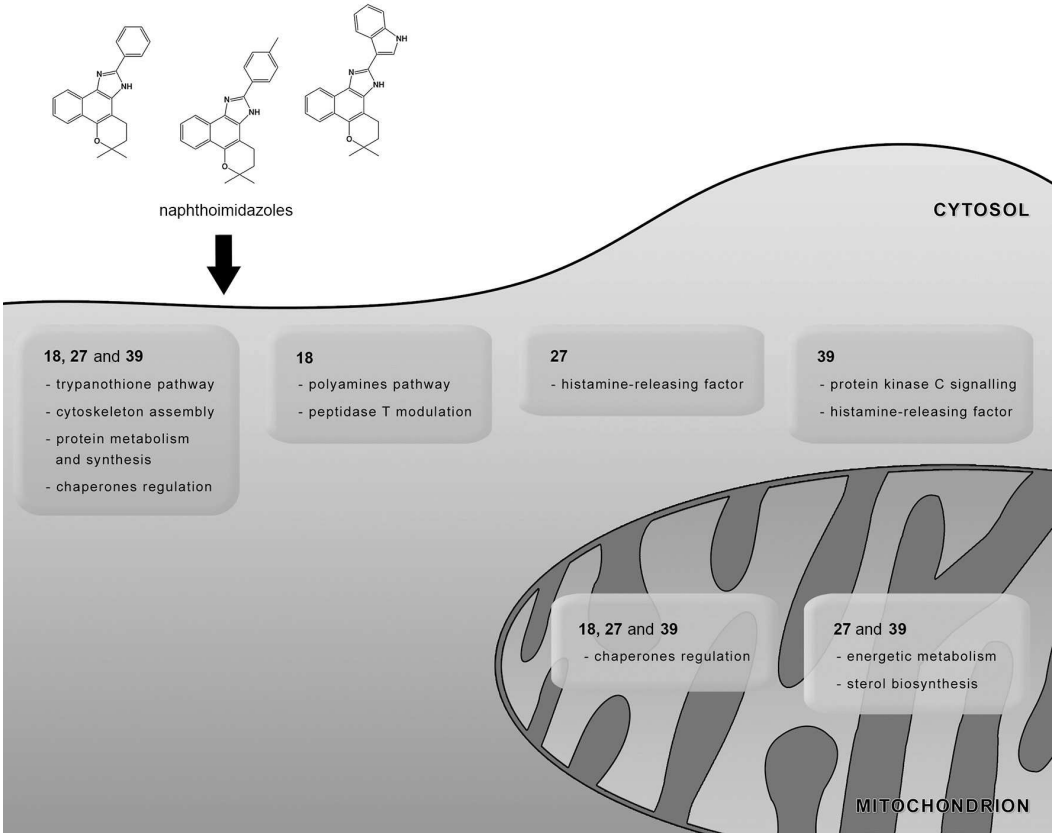


Figure 5. Similarities and differences among the mechanisms of action of each naphthoimidazole in *T. cruzi* epimastigotes. Most of the modulated proteins are mitochondrial proteins, indicating that this organelle is the main target of compounds **18**, **27** and **39**. These three compounds regulate the trypanothione pathway, cytoskeleton assembly, protein metabolism/synthesis and chaperone diversity. These alterations compromise different biological processes and lead to parasite death. Other proteins and/or pathways were also affected by the naphthoimidazoles including the polyamine pathway and peptidase T activity (**18**), ergosterol biosynthesis, energetic metabolism, histamine-releasing factor activity (**27** and **39**), and protein kinase C signalling (**39**).

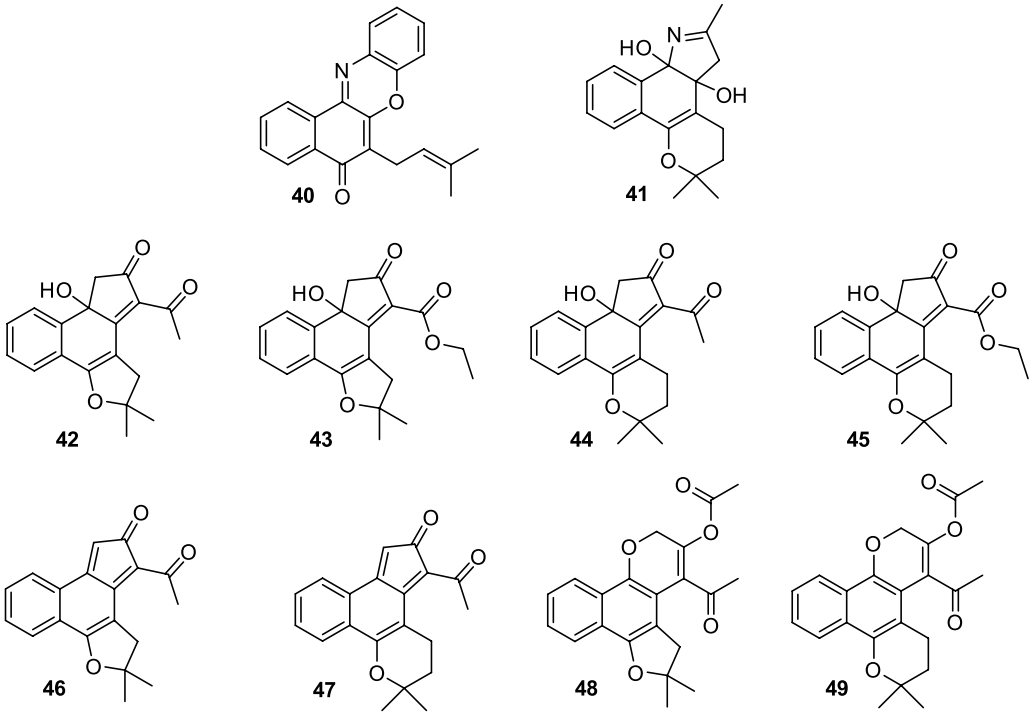


Figure 6. Heterocyclic compounds **40**-**49** obtained from lapachol (**1**), β-lapachone (**3**) and nor-β-lapachone (**5**).[67]

Table 3. Effects of the heterocyclic compounds **40**-**49** on *T. cruzi*

Compound	IC_{50}, 24 h / μM[a]
40	> 4000
41	1216.7 ± 349.1
42	nd[b]
43	> 4000
44	> 4000
45	56.1 ± 15.5
46	> 4000
47	786.9 ± 80.0
48	nd[b]
49	> 4000
Benznidazole	103.6 ± 0.6

[a]Mean ± standard deviation from three experiments performed in triplicate; [b]not determined.

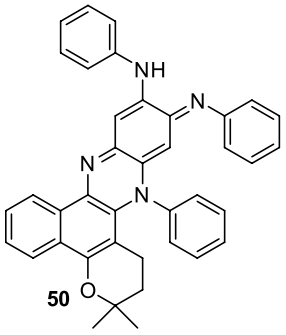


Figure 7. Phenazine derivative **50** obtained from β-lapachone (**3**).[75]

Cell cycle evaluations revealed a reduction in the number of parasites with duplicated genetic material, suggesting that the compound blocked cytokinesis. Transmission electron microscopy analyses of epimastigotes revealed the formation of well-developed endoplasmic reticulum profiles surrounding the reservosomes; these results suggest that there is close contact between both membranes. The

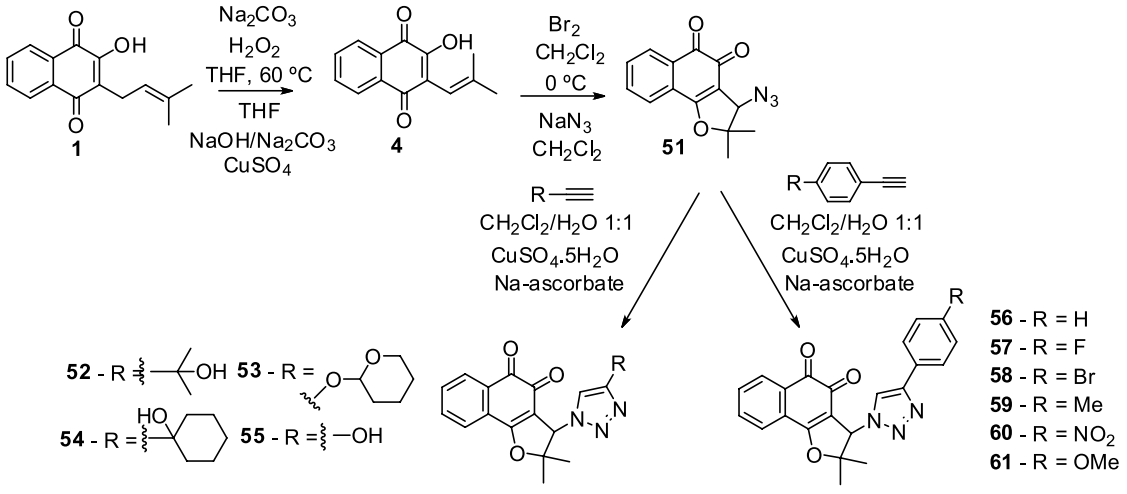
Table 4. Effects of nor-β-lapachone-based 1,2,3-triazoles on *T. cruzi*

Compound	IC_{50} / μM[a]
51	50.2 ± 3.8
52	151.9 ± 8.0
53	256.7 ± 38.7
54	57.8 ± 5.6
55	348.1 ± 44.2
56	17.3 ± 2.0
57	20.8 ± 1.9
58	101.5 ± 5.7
59	39.6 ± 4.0
60	21.8 ± 3.1
61	359.2 ± 11.1
Crystal violet	536.0 ± 3.0
Benznidazole	103.6 ± 0.6

[a]Mean ± standard deviation from three experiments performed in triplicate.

appearance of cytosolic concentric membrane structures was another morphological feature, suggesting that autophagy is a partial mode of action for compound **56**. Fluorescence microscopy analyses reinforced these data and indicated that a high percentage of MDC-labelled epimastigotes was present after treatment. Morphological damage in Golgi cisternae and blebbing of the flagellar membrane were also frequent alterations induced by this triazolic quinone. Interestingly, ultra-structural and flow cytometry studies showed that the mitochondrion was not affected by the treatment, suggesting that this organelle is not a target of compound **56**. The mechanism of action of this triazolic naphthofuranquinone differs from that of the other naphthoquinones studied because it involves autophagy (especially of the reservosomes) and cytokinesis impairment (Figure 8).[83]

Compound **56** was considered an important prototype for anti-*T. cruzi* activity, but its high level of cytotoxicity



Scheme 4. Nor-β-lapachone-based 1,2,3-triazoles **52**-**61**.[80,81]

Figure 8. Ultra-structural analysis of *T. cruzi* epimastigotes treated with compound **56**. (a) Transmission electron microscopy revealed reservosome disorganisation (R) and endoplasmic reticulum (ER) profiles in close contact with this organelle's membrane (black arrows). The nucleus and mitochondrion (M) exhibited typical morphologies. (b) Scanning electron microscopy examination revealed parasite body retraction (white thick arrows) and the impairment of mitosis (white arrowhead). Bar in (a): 0.2 μm. Bar in (b): 1 μm.

in mammalian cells was an impediment for further studies. We believed that it was necessary to structurally modify this compound to obtain a substance with a higher selectivity index (SI) that corresponds to the ratio LC_{50} (concentration that leads to damage of 50% of the mammalian cells)/IC_{50}. Another possibility would be to develop the compound within a controlled delivery system, which has been the focus of several studies aimed at solving drug toxicity issues. This important strategy can be used to optimise the therapeutic efficacy of the drug and reduce toxic side effects.[84] In Scheme 5, the naphthoquinoidal compounds designed to couple *ortho*-quinone to *para*-quinoidal structures are displayed. Our strategy was based on the combination of *ortho*- and *para*-quinoidal moieties that are able to generate high concentrations of reactive oxygen species, a property that is generally associated with the activity of this class of compounds. Based on the structural skeleton of compound **56**, compounds **62**-**64** were designed to preserve the main group, the quinoidal pharmacophore. Our approach proved to be effective, and compounds **62**, **63**, and **64** exhibited IC_{50} (24 h) values of 80.8, 6.8 and 8.2 μM, respectively (Table 5).[85] We were pleasantly surprised when heart muscle cell toxicity analyses produced LC_{50} (24 h) values of 63.1 and 281.6 μM for compounds **63** and **64**, respectively, which corresponded to SI of 9.3 and 34.3.[85]

Table 5. Effects of compounds **62**-**64** on *T. cruzi*

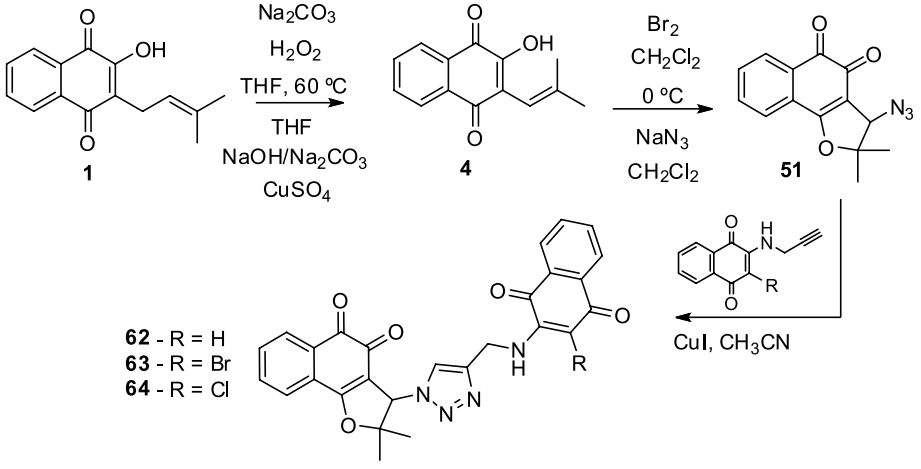
Compound	IC_{50} / μM[a]
62	80.8 ± 6.5
63	6.8 ± 0.7
64	8.2 ± 0.7
Benznidazole	103.6 ± 0.6
Crystal violet	536.0 ± 3.0

[a]Mean ± standard deviation from at least three experiments.

Aiming the establishment of a panel of minimum standardised procedures to advance leading compounds to clinical trials, the workshop Experimental Models in Drug Screening and Development for Chagas Disease was held in Rio de Janeiro (Brazil) organised by the Fiocruz Program for Research and Technological Development on Chagas Disease (PIDC) and DND*i*. During the meeting, the minimum steps, requirements and decision gates for the determination of the efficacy of lead compounds were evaluated by interdisciplinary experts and an *in vitro* and *in vivo* flowchart was designed to serve as a general and standardised protocol for drug screening.[86] Based on this flowchart and due to the high SI value attained, compound **64** will be assayed further for its effectiveness in *T. cruzi*-infected mice.

To obtain additional trypanocidal molecules with low toxicity in mammalian cells, new triazolic α- and nor-α-lapachones were synthesised and assayed for anti-*T. cruzi* activity based on a strategy we recently described involving C-ring modification.[87]

α-Lapachone-based 1,2,3-triazoles were synthesised as previously described (Scheme 6).[88] 4-Bromo-α-lapachone was prepared from α-lapachone (**2**) by obtaining a key



Scheme 5. Nor-β-lapachone 1,2,3-triazole coupled 1,4-naphthoquinones **62**-**64**.[85]

intermediate, 4-azido-α-lapachone (**65**). Using the click chemistry method,[89] several 1,2,3-triazoles **66**-**68** were synthesised. Unfortunately, this class of compounds was not active against trypomastigotes of *T. cruzi* and revealed IC_{50} (24 h) values greater than 500 μM for all derivatives.

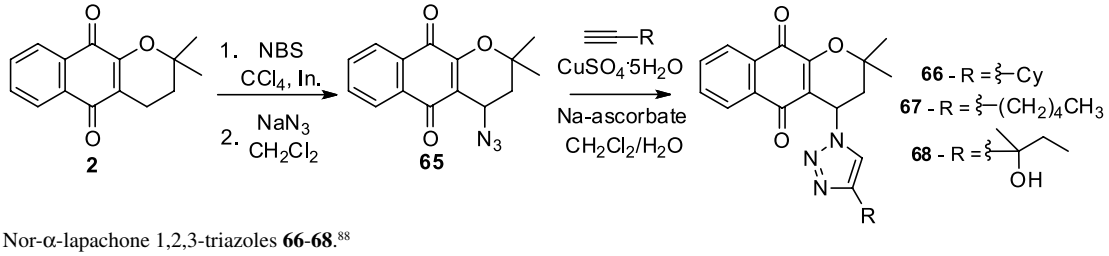
Using the same methodology with one minor difference (in this case, the initial compound used was nor-α-lapachone (**69**)), we prepared compounds **71**-**74** with high yields (Scheme 7). These substances were evaluated under the same conditions for anti-*T. cruzi* activity and were also found to be inactive.[85]

To structurally modify β-lapachone (**3**), C-ring modification[87] was used to synthesise compounds that were more active and selective towards *T. cruzi*. Thus, we described the insertion of 1,2,3-triazoles into compound **3**. The preparation of these derivatives was easily accomplished using the 3,4-dibromo-β-lapachone (**75**) obtained from compound **3**. After two steps, the key intermediate **77** was isolated and used to prepare β-lapachone-based 1,2,3-triazoles with moderate yields (Scheme 8).[90] These triazoles were evaluated against the trypomastigote form of *T. cruzi*, and all of the substances were more effective than crystal violet. When compared to Bz, compound **77** was 4 times more active than the standard drug and compound **81** exhibited similar activity (Table 6).[90]

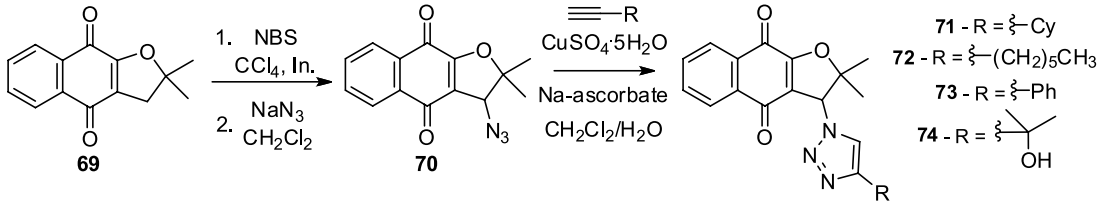
Table 6. Activity of β-lapachone-based 1,2,3-triazoles **78**-**81** on *T. cruzi*

Compound	IC_{50}, 24 h / μM[a]
76	248.3 ± 29.1
77	23.4 ± 3.8
78	313.0 ± 26.4
79	439.6 ± 31.6
80	219.8 ± 27.2
81	106.1 ± 19.0
Benznidazole	103.6 ± 0.6
Crystal violet	536.0 ± 3.0

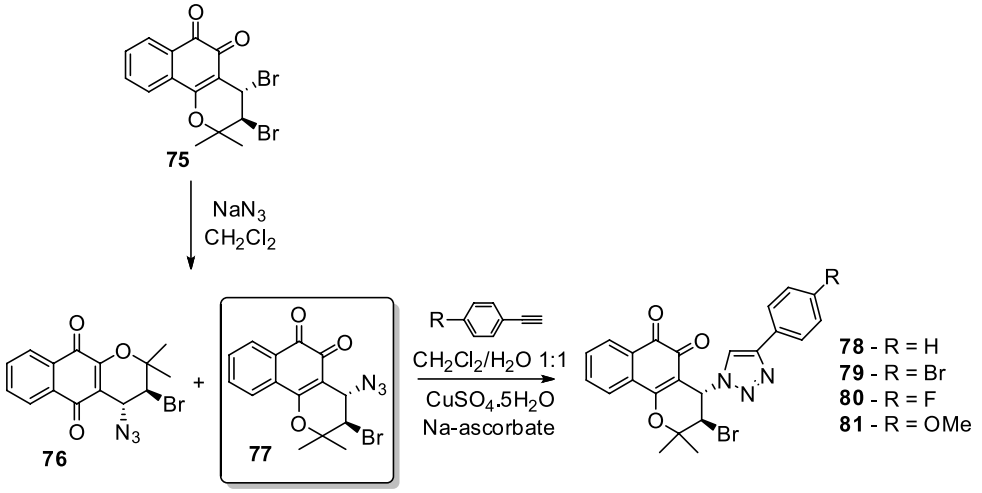
[a]Mean ± standard deviation from at least three experiments.



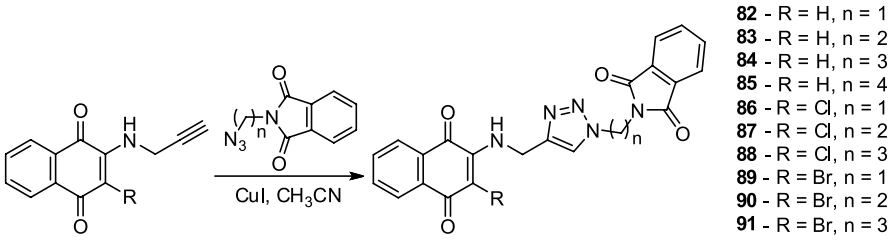
Scheme 6. Nor-α-lapachone 1,2,3-triazoles **66**-**68**.[88]



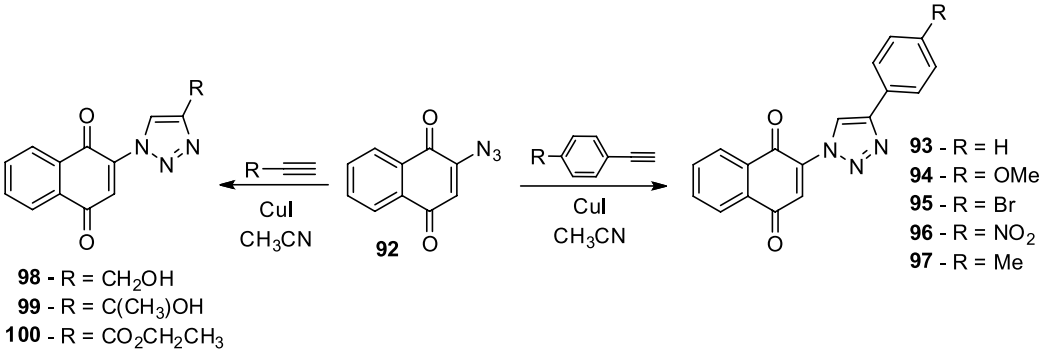
Scheme 7. Nor-α-lapachone-based 1,2,3-triazole **71**-**74**.[88]



Scheme 8. β-Lapachone-based 1,2,3-triazoles **78**-**81**.[90]



Scheme 9. 1,4-Naphthoquinone-derived 1,2,3-triazoles **82**-**91**.[85]



Scheme 10. Naphthoquinone-based 1,2,3-triazoles **93**-**100**.[91]

1,4-Naphthoquinone coupled to 1,2,3-triazole *N*-phthalimides (**82**-**91**) were recently prepared from brominated, chlorinated or unsubstituted quinones (Scheme 9).[85] Compounds **82**-**91** were inactive against *T. cruzi* and more studies regarding the mechanism of insertion of the 1,2,3-triazole ring into 1,4-naphthoquinone are necessary.

Meanwhile, 1,4-naphthoquinones with a direct insertion of a heterocyclic ring 1,2,3-triazole into the quinoidal structure were prepared, as shown in Scheme 10. Synthesis of the naphthoquinones coupled to 1,2,3-triazoles was initially reported by Nascimento *et al.* (Scheme 10).[91] In assays with trypomastigote forms of *T. cruzi*, the most active substances displayed IC_{50} values in the range of 10.9 to 80.2 μM (Table 7).[85] Compounds **93** and **98** exhibited IC_{50} values of 10.9 and 17.7 μM, respectively, and are thus very promising structures. Further studies regarding their mechanism of action, cytotoxicity levels and *in vivo* activity are therefore necessary. It is important to note that the *para*-naphthoquinone 1,2,3-triazoles are easily obtained in only two steps from the starting material 1,4-naphthoquinone and both reactions have good to excellent yields.

Table 7. Effects of naphthoquinone-based 1,2,3-triazoles **93**-**100** on *T. cruzi*

Compound	IC_{50} / μM[a]
93	10.9 ± 1.8
94	45.8 ± 5.1
95	492.2 ± 17.5
96	2005.7 ± 9.9
97	113.1 ± 5.7
98	17.7 ± 3.1
99	80.2 ± 5.4
100	67.6 ± 7.7
Benznidazole	103.6 ± 0.6
Crystal violet	536.0 ± 3.0

[a]Mean ± standard deviation from three experiments performed in triplicate.

Using the methodology described by the Pinto group,[92] we prepared substituted nor-β-lapachones arylamino from nor-lapachol (**4**) at high yields (Figure 9), and these compounds were evaluated for anti-*T. cruzi* activity (Table 8).[93,94] The trypanocidal activity of compounds **103**, **108**, **110**, and **112**-**114** was higher than that of Bz, a drug commonly used to combat *T. cruzi* infections. Compound **112**, which contained the bromine atoms, was the most active compound and exhibited an IC_{50} value of 24.9 μM.

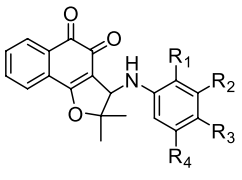


Figure 9. Nor-β-lapachone arylamino substituted compounds **101**-**116**.[93,94]

Table 8. Activity of nor-β-lapachone arylamino substituted compounds **101**-**116** on *T. cruzi*

Compounds	IC_{50} / μM[a]
101	332.8 ± 23.3
102	140.8 ± 11.9
103	86.3 ± 4.6
104	> 4000
105	384.4 ± 52.5
106	952.5 ± 71.1
107	857.3 ± 96.4
108	88.2 ± 6.7
109	2517.9 ± 169.8
110	55.6 ± 4.6
111	1756.1 ± 91.8
112	24.9 ± 7.4
113	43.8 ± 7.4
114	59.6 ± 13.2
115	526.2 ± 80.5
116	156.2 ± 9.1
Benznidazole	103.6 ± 0.6

[a]Mean ± standard deviation from three experiments performed in triplicate.

These structures represent an important starting point for the attainment of new trypanocidal compounds.

In a previous work,[92] Silva *et al.* described the synthesis of derivatives obtained from C-allyl lawsone, as shown in Scheme 11. These compounds exhibited activity against *T. cruzi* in both the bloodstream trypomastigote and epimastigote forms (Tables 9 and 10). The effects of compounds **117**-**119** on epimastigote proliferation were monitored for up to 4 days.

Compounds **117**-**119** derived from C-allyl lawsone were effective against the three forms of the parasite, and the intracellular amastigote was the most susceptible form.[95] Transmission electron microscopy examination of treated epimastigotes and bloodstream trypomastigotes revealed a drastic mitochondrial swelling with a washed-out matrix profile. Potent dose-dependent collapse of the mitochondrial membrane potential revealed by rhodamine 123 staining together with an inhibition of mitochondrial complex I-III activities and a reduction in succinate-induced oxygen consumption strongly corroborated the central role of the mitochondrion in these compounds' mechanisms of action. Moreover, an

Table 9. Effects of the naphthoquinones **117**-**119** on *T. cruzi*

Compounds	IC_{50} / μM[a]
117	641 ± 38
118	398 ± 56
119	158 ± 9
Benznidazole	103.6 ± 0.6

[a]Mean ± standard deviation from three experiments performed in triplicate.

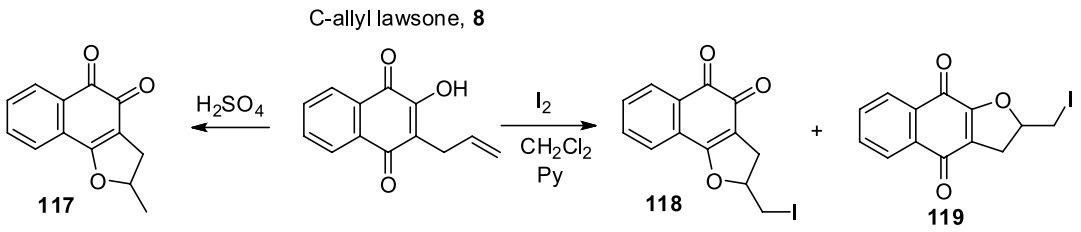
Table 10. Effects of the naphthoquinones **117**-**119** on epimastigote forms of *T. cruzi* (in μM)

Compounds	IC_{50}, 1 day	IC_{50}, 2 day	IC_{50}, 3 day	IC_{50}, 4 day
117	13.2 ± 2.2	12.4 ± 1.4	11.7 ± 1.5	12.7 ± 2.0
118	24.9 ± 1.8	21.8 ± 2.4	19.5 ± 2.4	18.3 ± 4.9
119	7.9 ± 1.3[a]	3.7 ± 0.3	3.0 ± 0.7	2.6 ± 0.3

[a]Mean ± standard deviation from three independent experiments.

increase in the production of hydrogen peroxide by this organelle in treated epimastigotes was also observed. However, some differences in the mode of action of naphthofuranquinones were apparent in epimastigotes and trypomastigotes. In the insect form, the trypanocidal effects of the compounds were a consequence of the parasite redox balance modulation, whereas in the bloodstream form, mitochondrial dysfunction affected energy transduction reactions, which compromised the protozoa's bioenergy efficiency. Naphthoquinones interfere with electron flow at the inner mitochondrial membrane by diverting electrons away from ubiquinone. The oxidation of semiquinones back to quinones leads to the generation of reactive oxygen species that compromise the activity of complex I-III and oxygen consumption capability, which culminates in parasite death.[95]

In another set of experiments, the trypanocidal activity of sixteen 1,4-naphthoquinones was assessed on both *T. cruzi* trypomastigotes and epimastigotes (Figure 10 and Table 11).[96] In the case of the naphthoquinones **120**-**134**, different assay conditions were used to analyse the effects on trypomastigotes. While all of the previous experiments were performed in the presence of 5% mouse blood and at 4 °C (Bz IC_{50} = 103.6 ± 0.6 μM) as previously mentioned, the present compounds were assayed at 37 °C in absence of blood (Bz IC_{50}= 26.0 ± 4.0 μM).



Scheme 11. Synthetic route for the attainment of methylated and iodinated naphthoquinones **117**-**119**.[92]

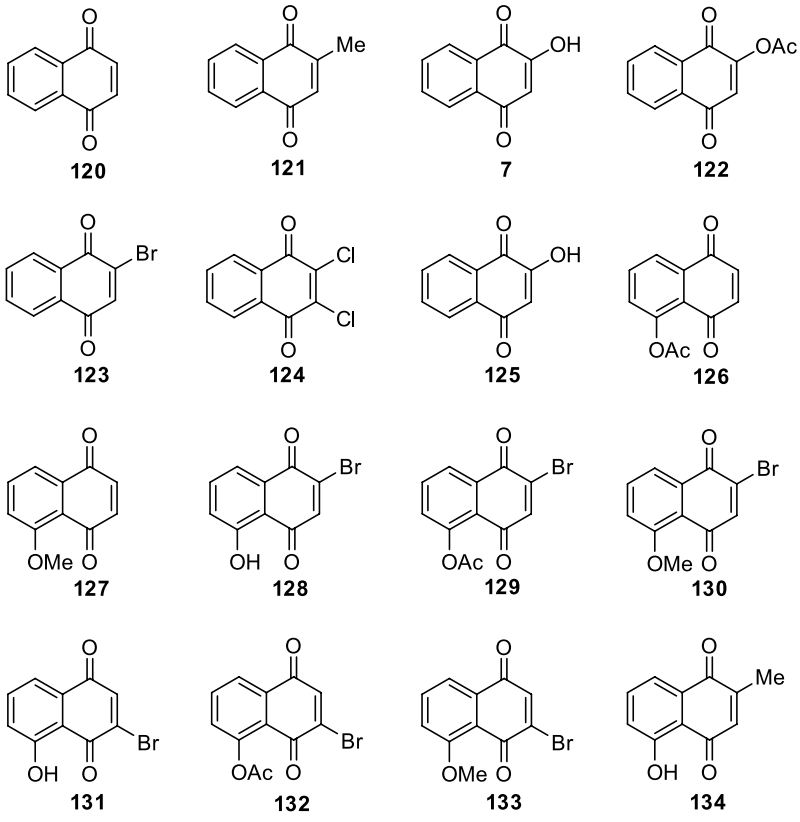


Figure 10. Naphthoquinones **120**-**134** and lawsone (**7**).[96]

Table 11. Effects of the naphthoquinones **120**-**134** on *T. cruzi* at 37 °C

Compound	IC_{50} / μM[a]
120	0.79 ± 0.02
121	6.04 ± 0.35
122	63.02 ± 5.8
123	1.37 ± 0.03
124	2.17 ± 0.29
125	6.51 ± 0.48
126	0.16 ± 0.01
127	1.02 ± 0.29
128	2.15 ± 0.22
129	2.43 ± 0.50
130	1.25 ± 0.26
131	2.52 ± 0.37
132	0.85 ± 0.08
133	1.41 ± 0.15
134	1.38 ± 0.26
7	563.18 ± 83.28
Benznidazole	26.0 ± 4.0

[a]Mean ± standard deviation from three experiments performed in triplicate.

Four compounds were selected from this series for mode of action studies: the prototype naphthoquinone **120** and three juglone derivatives (**126**, **127** and **130**).[96] These four compounds were effective against parasite proliferative forms (epimastigotes and intracellular amastigotes) and reduced the infection of peritoneal macrophages and heart muscle cells. Ultra-structural studies of treated epimastigotes suggested that the mitochondrion are a primary target, due to the apparent swelling of the organelle and the appearance of membranous structures in its matrix (Figure 11). Mitochondrial membrane potential was evaluated by tetramethylrhodamine ethyl ester (TMRE) labelling, and all four quinones induced a depolarisation of this organelle, which reduced the intensity of TMRE fluorescence by up to 50%. Since an uncoupled mitochondrion generates reactive oxygen species (ROS), ROS production can be examined by DHE labelling; only compound **126** led to a discrete increase in the percentage of DHE+ epimastigotes. Mechanistically, it was reasonable to postulate that the collapse of the mitochondrial potential was mediated by ROS generation in the treated parasites. The absence of oxidative stress induced by compounds **120**, **127** and **130** could be attributable to the involvement of more than one mode of action in the trypanocidal activity of these compounds, leaving ROS generation suppressed by the detoxification system of the parasite. The intense redox activity of compound **126** could be attributed to the acetyl group present in its structure that facilitates quinone reduction. Furthermore, other morphological alterations were described, such as atypical cytosolic membranous structures and the appearance of

endoplasmic reticulum surrounding reservosomes, which is indicative of autophagy. In addition, intense cytosolic vacuolisation, the formation of blebs in the flagellar membrane and the loss of cytosolic electron-density were also observed. The ultra-structural autophagic evidence suggests that the endoplasmic reticulum participates in the observed pre-autophagosomal formation.[96]

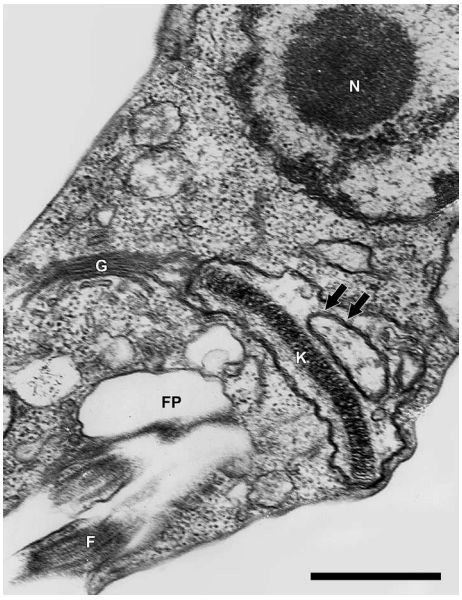


Figure 11. Transmission electron microscopy analysis of a *T. cruzi* epimastigote treated with compound **130**. The treatment induced the appearance of membranous structures inside the mitochondrion (black thick arrows). N: nucleus; G: Golgi; FP: flagellar pocket; F: flagellum; K: kinetoplast. Bar: 0.5 μm.

3. Conclusions

This review describes our efforts to develop an effective trypanocidal drug. Synthesis procedures and biological data regarding anti-*T. cruzi* activity were described and studies of the mechanism of action of these compounds were detailed to provide an overview of the progress made by our research group in collaboration with several researchers around the world. Among the quinones and derivatives investigated, naphthoimidazoles derived from β-lapachone presented promising biological activity together with low toxicity to the host cells, opening interesting perspectives for their investigation *in vivo*. On the other hand, naphthoquinones presenting different moieties in their structures showed distinct modes of action. It is well-known that quinones induce ROS production also in *T. cruzi*. Our previous data pointed to ROS generation as part of the naphtoquinones' mechanism of action and the central role of the parasite mitochondrion, depending on the moiety linked to the quinoidal ring. In this scenario, as an example, a triazolic naphthoquinone led to discrete increase in ROS levels and did not compromise the mitochondrial functionality as well. The naphthofuranquinone and juglone derivatives strongly affected this organelle physiology interfering with the oxygen uptake and mitochondrial membrane potential. High amounts of ROS were produced by the mitochondrion of treated parasites culminating in *T. cruzi* death. Notwithstanding, many questions still remain unanswered about the molecular mechanisms involved in the trypanocidal effect of these compounds and their selectivity for different cellular structures in the protozoa, we hope that this review contributes to the development of new candidates for Chagas disease.

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Eufrânio N. da Silva Júnior received his degree in chemistry from the Catholic University of Brasília (UCB). In 2007, he completed his MSc at the Fluminense Federal University (UFF) and in 2009 he concluded his PhD at the University of Brasilia (UnB). In 2010, he became Professor of Chemistry at the Federal University of Minas Gerais (UFMG). His research interests are focused on click chemistry reactions, asymmetric organocatalysis and on the synthesis of heterocyclic and naphthoquinoidal bioactive compounds. Currently, he is also interested in obtaining fluorescent substances for the study of pharmacological and DNA-binding properties.

Guilherme A. M. Jardim received his degree in Chemistry from the Federal University of Minas Gerais (UFMG). He is currently pursuing his MSc at the same university under the supervision of Prof Eufrânio N. da Silva Júnior. His dissertation work is focused largely

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Solange L. de Castro received her degree in Industrial Chemistry from the Federal University of Rio de Janeiro (UFRJ). In 1991, she completed her PhD at the Oswaldo Cruz Institute (FIOCRUZ) in experimental chemotherapy of Chagas disease. She is a senior researcher at FIOCRUZ. Her research interests are focused on chemotherapy, with special interest in the studies about the trypanocidal activity and mechanism of action of naphthoquinones and derivatives.

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