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Reaction of naphthoquinones with substituted nitromethanes. Facile synthesis and antifungal activity of naphtho[2,3-*d*]isoxazole-4,9-diones

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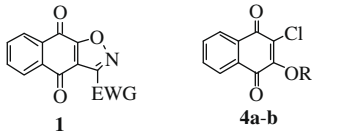
ABSTRACT

We report here a simple entry into naphtho[2,3-*d*]isoxazole-4,9-dione system containing a EWG in position 3 using the readily available 2,3-dichloro-1,4-naphthoquinone and nitromethyl derivatives in the presence of base. Antifungal activity of synthesised naphthoquinones was evaluated against ATCC and PYCC reference strains of *Candida*. The results suggest that the naphtho[2,3-*d*]isoxazole-4,9-dione scaffold has the potential to be developed into novel and safe therapeutic antifungal agents.

Compounds containing the quinone group represent an important class of biologically active molecules that are widespread in nature.[1] Isoxazoles also have a significant number of biological applications, displaying hypoglycemic, analgesic, anti-inflammatory, antifungal, anti-bacterial and HIV-inhibitory activity.[2] Naphtho[2,3-*d*]isoxazole-4,9-diones **1** (Fig. 1) combine both functionalities and are an attractive target. Importantly, compounds **1** also display agricultural applications.[3]

To date, most of the routes to naphtho[2,3-*d*]isoxazole-4,9-diones described in the literature use *in situ* generation of nitrile oxides, require the synthesis of the necessary starting materials and do not allow the introduction of electron-withdrawing group (EWG) in position 3.[4–6] To the best of our knowledge, there is only one route that introduces an ethyl ester in position 3 of the isoxazole moiety that involves a manganese(III) acetate initiated reaction between readily available 1,4-naphthoquinones and ethyl nitroacetate. This method is highly wasteful, requiring 8 equiv of ethyl nitroacetate and 12 equiv of manganese(III) acetate.[7,8]

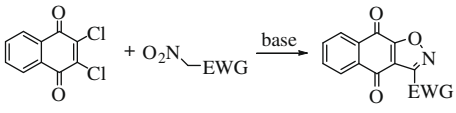
Recently we described naphtho[2,3-*d*]isoxazole-4,9-dione-3-carboxylates **1a–b** as potent, non-cytotoxic, antiapoptotic agents, through modulation of apoptotic pathways.[9] The two compounds were synthesized from 1 equiv of each commercially available starting material, 2,3-dichloro-1,4-naphthoquinone **2** and a nitromethyl derivative **3a–b**, in the presence of base (Scheme 1). By this method, the isoxazole **1a** was obtained in 22% yield (35% based on recovered **2**) after 3 d at reflux in the presence of NEt_3 in ethanol. Using methyl nitroacetate **3b** the isoxazole **1b** was obtained in 19% yield, along with the isoxazole **1a** (7% yield) due to ester exchange, and 2-chloro-3-ethoxy-1,4-naphthoquinone **4a** (13% yield). To avoid the problem of ester exchange, we changed the solvent to MeOH but did not observe any formation of isoxazole after 3 d at reflux (Table 1). In this case, we only isolated



1 **4a-b**

a R=Et; **b** R=Me

Figure 1. Structure of compounds **1** and **4**.



2 **3a-d** **1a-d**

a EWG=CO_2Et; **b** EWG=CO_2Me; **c** EWG=SO_2Ph; **d** EWG=COPh

Scheme 1. Synthesis of naphtho[2,3-*d*]isoxazole-4,9-diones **1a–d**.

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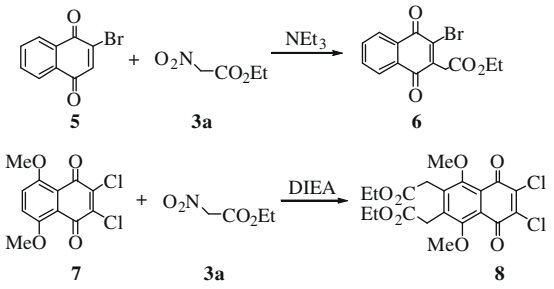
Table 1
Reaction conditions used for the synthesis of compounds **1a–d**, **6** and **8**

Starting materials		Solvent	Base	Reflux (h)	Product (yield %)
2	**3a**	EtOH	DIEA	6.5	**1a** (33) **4a** (11)
5		EtOH	NEt_3	6.5	**6** (78)
		EtOH	NEt_3	29.0	**1a** (13) **6** (26)
7		EtOH	DIEA	1.5	**8** (56)
		EtOH	DIEA	30.0	**8** (37)
2	**3b**	EtOH	DIEA	6.5	**1b** (33) **4a** (12)
		MeOH	NEt_3	72.0	**4b** (33)
2	**3c**	EtOH	DIEA	96.0	**4a** (15)
		CH_3CN	NEt_3	4.0	**1c** (10)
		CH_3CN	DIEA	2.0	—
		CH_3CN	K_2CO_3	48.0	—
2	**3d**	EtOH	DIEA	24.0	**4a** (41)
		CH_3CN	DABCO	6.0	**1d** (12)
		CH_3CN	NEt_3	5.0	**1d** (32)
		CH_3CN	DIEA	4.5	**1d** (32)
		CH_3CN	K_2CO_3	5.0	**1d** (23)



Scheme 2. Reactions of 2-bromo-1,4-naphthoquinone **5** and 2,3-dichloro-5,8-dimetoxi-1,4-naphthoquinone **7** with **3a**.

2-chloro-3-methoxy-1,4-naphthoquinone **4b** (33% yield)—presumably due to the lower temperature of reflux.

We then decided to continue working on this new methodology in order to improve the low yields of naphtho[2,3-*d*]isoxazole-4,9-dione-3-carboxylates **1a–b**. An improved synthesis was accomplished, using diisopropylethylamine (2 equiv) as base, in the presence of molecular sieves.[10a] In this case, the reaction proceeded faster with no starting material being recovered; after 6.5 h we isolated isoxazole **1a** (33% yield), along with 2-chloro-3-ethoxy-1,4-naphthoquinone **4a**[11] (11% yield) formed by substitution of a Cl atom by the solvent. Using methyl nitroacetate **3b** the isoxazole **1b** was obtained in 33% yield, along with 2-chloro-3-ethoxy-1,4-naphthoquinone **4a** (12% yield).[10a]

When 2-bromo-1,4-naphthoquinone **5** was used as starting material, naphthoquinone **6** (78% yield) was obtained from reaction with **3a**, after 6.5 h at reflux in the presence of NEt_3 in ethanol (Scheme 2). Only after 29 h at reflux, was the isoxazole **1a** obtained (13% yield), along with the naphthoquinone **6** (26% yield).[12,13] The latter obviously arises from Michael addition of the nitronate anion at the least substituted quinone carbon atom followed by loss of the nitrite ion. Attack at the C–H carbon is presumably preferred because (i) this centre is less sterically hindered, and (ii) bromine stabilizes an adjacent anion better than does an H atom.

When we used 2,3-dichloro-5,8-dimethoxy-1,4-naphthoquinone **7** and **3a** as starting materials, after 1.5 h at reflux in ethanol in the presence of 2 equiv of DIEA and molecular sieves, we isolated compound **8** in 56% yield (Scheme 2).[14] In this case, no formation of isoxazole was observed, even after 30 h at reflux in the presence of NEt_3 (Table 1).

This method seems to be general for other nitromethyl derivatives, as it was also possible to obtain naphtho[2,3-*d*]isoxazole-4,9-diones with a sulfone **1c** and a ketone **1d** in position 3 (Scheme 1). When reaction of **2** was attempted with phenylsulfonylnitromethane **3c** or benzoylnitromethane **3d** in the presence of DIEA and ethanol, only 2-chloro-3-ethoxy-1,4-naphthoquinone **4a**, from reaction of the quinone with the solvent, could be isolated (Table 1).[10a] However, when we changed the solvent to acetonitrile, it was then possible to obtain the isoxazoles **1c** and **1d**, respectively (Table 1).[10b] Isoxazole **1c** was only formed in the presence of NEt_3 (10% yield). In the presence of DIEA or K_2CO_3 a complex mixture of products was obtained. Isoxazole **1d** however was obtained using as bases DABCO (12% yield), NEt_3 (32% yield), DIEA (32% yield) or K_2CO_3 (23% yield). To try to improve the yield of **1d**, we decided to study the effect of using 2 equiv of nitroacetate **3d**, however in the presence of NEt_3 in CH_3CN, we obtained a more complex mixture, and we could only isolate isoxazole **1d** in 14% yield.

In all the above reactions, a complex mixture of products was obtained, and we could only isolate as the major products the products described in Table 1.

Structural assignment of products **1** was based on spectroscopic data,[15,16] which accorded with the literature.[7] Confirmation of the isoxazole structure **1** came from the X-ray crystallographic analysis.[17]

Naphtho[2,3-*d*]isoxazole-4,9-diones **1a–d** and naphthoquinones **4a–b**, **6** and **8** were evaluated for their antifungal activity against various ATCC and PYCC reference strains of *Candida*.[18] The MIC_{50} values were calculated using probit analysis based on triplicate assays and are presented in Table 2. All compounds showed a reasonable level of antifungal activity at the μg/mL level, ranging from the lowest MIC_{50} value of 0.2 μg/mL for compound **1a** against PYCC 2545 (*Candida parapsilosis*) and PYCC 2418^T (*Candida glabrata*) to the highest of 47.9 μg/mL for compound **4a** against ATCC 90028 (*Candida albicans*).

Inspection of the data in Table 2, allows the following observations to be made:

Table 2
Determination of MIC_{50} μg/mL (μM) for compounds **1a–d**, **4a–b**, **6** and **8**

Compound	*C. albicans* ATCC 90028	*C. albicans* PYCC 3436^T	*C. krusei* PYCC 3341	*C. parapsilosis* PYCC 2545	*C. parapsilosis* ATCC 22019	*C. glabrata* PYCC 2418^T
1a	3.1 (11.5)	0.5 (1.8)	0.6 (2.3)	0.2 (0.6)	0.5 (1.7)	0.2 (0.6)
1b	3.1 (12.2)	0.2 (0.7)	0.6 (2.4)	0.3 (1.3)	0.5 (1.8)	0.7 (2.6)
1c	19.2 (56.7)	0.8 (2.5)	3.3 (9.7)	0.5 (1.6)	2.1 (6.1)	0.5 (1.5)
1d	21.6 (71.2)	0.5 (1.6)	6.4 (21.2)	4.0 (13.2)	1.0 (3.3)	14.4 (47.6)
4a	47.9 (202.4)	2.3 (9.5)	0.9 (3.9)	11.4 (48.3)	9.6 (40.4)	0.9 (3.8)
4b	16.5 (73.9)	2.3 (10.4)	1.0 (4.6)	1.2 (5.3)	1.0 (4.7)	3.7 (16.7)
6	1.5 (4.5)	0.3 (0.9)	nd[a]	0.4 (1.1)	1.0 (3.2)	3.9 (12.0)
8	25.7 (56.0)	5.6 (12.2)	3.0 (6.5)	7.0 (15.2)	8.4 (18.4)	2.3 (5.1)
AmpB	1.1 (1.2)	0.5 (0.5)	0.9 (1.0)	0.4 (0.5)	0.4 (0.5)	0.3 (0.4)

[a] Not determinate due to absence of yeast growth in all bioassays.

(1) There was marked variability among the strains with regard to their susceptibilities to the various test compounds. This is particularly evident for the *C. albicans* ATCC 90028 and PYCC 3436T strains (e.g., compounds **1c**, **1d** and **4a**). Such variability suggests there are biological factor(s) affecting strain/species and test compound bioactivity.
(2) Strain ATCC 90028 appeared to be the least sensitive, to almost all the compounds. Only compound **6**, with an MIC_{50} of 1.5 μg/mL demonstrated antifungal activity equivalent to that of AmpB (Amphotericin B) for this strain. In addition, this compound also had notable activity against PYCC strains 2545 and 3436^T.
(3) Only compounds **1a** and **1b** showed a level of activity equivalent to that of AmpB against strain ATCC 22019. Compound **1a** appeared to have the broadest antifungal activity, showing MIC_{50} values at or below that of AmpB for all strains, except ATCC 90028. Compound **1b** was next in breadth of activity, with MIC_{50} values at or below those of AmpB for four of the six strains. Compound **1c** demonstrated reasonable antifungal activity, with MIC_{50} levels <1.0 μg/mL against PYCC strains, 2545, 3436^T and 2418^T.
(4) The order of the antifungal activity in compounds **1** seems to depend on the nature of the EWG in position 3 and varies in the order ester > sulfone > ketone.
(5) Compounds **1d**, **4a** and **4b**, also showed reasonable antifungal activities equivalent to that of AmpB against one strain, each. Compound **8**, although exhibiting fair antifungal activity at the μg/mL level against all strains, did not present any MIC_{50} values at or below that of AmpB against any of the strains.

In summary, we describe a new methodology for the synthesis of isoxazoles **1** containing an EWG in position 3. Although the yields are lower than those described for the synthesis of isoxazole **1a** (lit. 55% yield),[7] our procedure uses cleaner chemistry and only 1 equiv of both commercial starting materials. Also, our method is not restricted to the use of ethyl nitroacetate, but seems to be general for other nitromethyl derivatives. In addition, all the test compounds showed effective levels of antifungal activity at the μg/mL level. Importantly, **1a** and **1b** showed the broadest activity and were equivalent to or better than the standard antifungal drug Amphotericin B against five of the six strains examined. The observation that naphtho[2,3-*d*]isoxazole-4,9-dione-3-carboxylates **1a–b** are non-cytotoxic in human cellular lines[9] strongly suggests that the naphtho[2,3-*d*]isoxazole-4,9-dione scaffold has the potential to be developed into novel and safe therapeutic antifungal agents. The mechanism for the formation of isoxazoles **1a–d** is understudy.

Acknowledgments

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References and notes

1. Dewick, P. M., In *Medicinal Natural Products*, 2nd ed., Wiley, Chichester, UK, 2002
2. Pinho e Melo, T. M. V. D. *Curr. Org. Chem.* **2005**, *9*, 925.
3. (a) Kano, H.; Ogata, M.; Yukinaga, H. U.S. Patent 3,835,168, 1974.; (b) Kano, H.; Ogata, M.; Yukinaga, H. U.S. Patent 3,933,828, 1976.
4. Alguacil, R.; Fariña, F.; Martín, M. V.; Paredes, M. C. *Tetrahedron Lett.* **1995**, *36*, 6773.
5. Stevens, J.; Welton, T.; Deville, J.; Behar, V. *Tetrahedron Lett.* **2003**, *44*, 8901.
6. Matsuura, T.; Bode, J. W.; Hachisu, Y.; Suzuki, K. *Synlett* **2003**, 1746.
7. Chuang, C.; Wu, Y.; Jiang, M. *Tetrahedron* **1999**, *55*, 11229.
8. Snider, B. B.; Che, Q. *Tetrahedron* **2002**, *58*, 7821.
9. Santos, D.; Santos, M. M. M.; Viana, R. J. S.; Castro, R. E.; Moreira, R.; Rodrigues, C. M. P. *Chem. Biol. Interact.* **2009**, *180*, 175.
10. (a) Nitromethyl derivative (2.2 mmol) and DIEA (4.4 mmol) were added to a solution of 2,3-dichloro-1,4-naphthoquinone (2.2 mmol) in EtOH (50 mL) with molecular sieves. After reflux, under argon atmosphere, the solution was filtered and evaporated to dryness. The residue was submitted to flash chromatography (*n*-hexane/ethyl acetate 8:2).; (b) Nitromethyl derivative (2.2 mmol) and base (4.4 mmol) were added to a solution of 2,3-dichloro-1,4-naphthoquinone (2.2 mmol) in CH_3CN (50 mL). After reflux, under argon atmosphere, the mixture was diluted with ethyl acetate and washed with H_2O (3×). The organic extract was dried and concentrated. The residue was submitted to flash chromatography (*n*-hexane/ethyl acetate 8:2).
11. Fieser, L. F.; Brown, R. H. *J. Am. Chem. Soc.* **1949**, *71*, 3609.
12. *Compound* **6**: mp 122–124 °C; IR (NaCl) 1721, 1674; δ_H (400 MHz, $CDCl_3$) 1.26 (3H, t, J = 8.0 Hz, CH_3), 3.92 (2H, s, CH_2), 4.18 (2H, q, J = 8.0 Hz, OCH_2), 7.76 (2H, t, J = 4.0 Hz, ArH), 8.10–8.13 (1H, m, ArH), 8.15–8.18 (1H, m, ArH); δ_C (100 MHz, $CDCl_3$) 14.1 (CH_3), 37.1 (CH_2), 61.6 (OCH_2), 127.3 (CHAr), 127.7 (CHAr), 131.1 (CAr), 131.2 (CAr), 134.2 (CHAr), 134.4 (CHAr), 141.2 (CAr), 144.7 (CAr), 168.1 (CO), 177.4 (CO), 181.1 (CO).
13. McElvain, S. M.; Engelhardt, E. L. *J. Am. Chem. Soc.* **1944**, *66*, 1077.
14. *Compound* **8**: mp 134–135 °C; IR (NaCl) 1723, 1661; δ_H (400 MHz, $CDCl_3$) 1.25 (3H, t, J = 8.0 Hz, CH_3), 3.65 (2H, s, CH_2), 3.93 (3H, s, OMe), 4.15 (2H, *q*, J = 8.0 Hz, OCH_2); δ_C (100 MHz, $CDCl_3$) 14.1 (CH_3), 32.9 (CH_2), 61.6 (OCH_2), 62.0 (OCH_3), 124.4 (CAr), 136.7 (CAr), 141.6 (CAr), 153.2 (CAr), 169.0 (CO), 182.1 (CO).
15. *Compound* **1c**: mp 190–191 °C; IR (NaCl) 1716, 1675; δ_H (400 MHz, $CDCl_3$) 7.67 (2H, t, J = 8.0 Hz, ArH), 7.78 (1H, t, J = 8.0 Hz, ArH), 7.83–7.92 (2H, m, ArH), 8.24–8.30 (4H, m, ArH); δ_C (100 MHz, $CDCl_3$) 118.9 (CAr), 127.7 (CHAr), 128.1 (CHAr), 129.5 (2 CHAr), 129.9 (2 CHAr), 131.5 (CAr), 133.0 (CAr), 134.3 (CHAr), 135.5 (CHAr), 135.8 (CHAr), 137.3 (CAr), 162.5 (CAr), 166.6 (CN), 172.3 (CO), 175.7 (CO). HMRS $C_{17}H_{10}NO_5S$ (M+1) 340.02797, found 340.02847.
16. *Compound* **1d**: mp 124–125 °C; IR (NaCl) 1689, 1589; δ_H (400 MHz, $CDCl_3$) 7.56 (2H, t, J = 8.0 Hz, ArH), 7.69–7.73 (1H, m, ArH), 7.84–7.89 (2H, m, ArH), 8.08 (2H, d, J = 8.0 Hz, ArH), 8.21–8.23 (1H, m, ArH), 8.30–8.32 (1H, m, ArH); δ_C (100 MHz, $CDCl_3$) 121.2 (CAr), 127.7 (CHAr), 127.8 (CHAr), 129.0 (2 CHAr), 130.5 (2 CHAr), 132.0 (CAr), 133.3 (CAr), 134.7 (CHAr), 134.9 (CAr), 135.3 (CHAr), 135.5 (CHAr), 157.8 (CAr), 165.3 (CN), 177.0 (CO), 177.1 (CO), 188.9 (CO). HMRS $C_{18}H_{10}NO_4$ (M+1) 304.06098, found 304.06211.
17. Crystal data for $C_{14}H_9NO_5$, M_r = 271.22, triclinic space group $P\bar{1}$, a = 7.1485(3), b = 8.5516(2), c = 10.1860(5) Å, α = 79.315(2), β = 88.841(2), γ = 76.382(2)°, U = 594.50(4) Å^3, Z = 2, D_{calcd} = 1.515 g cm^{-3}, μ = 0.117 mm^{-1}, F(0 0 0) = 280, pale yellow crystal 0.6 × 0.4 × 0.05 mm, θ_{max} = 27.49°, 2704 unique data, 10685 measured (R_{int} = 0.0272), R_1 = 0.0363 and wR_2 = 0.0988 [$I > 2\sigma$ (I)]. Supplementary data have been deposited with the CCDC deposition number CCDC 674974.
18. Determination of MIC Levels: Antifungal activities of **1a–d**, **4a–b**, **6** and **8** and amphotericin B (AmpB; Sigma–Aldrich, Sigma A2411), were based on Minimal Inhibitory concentrations for 50% cell death (MIC_{50}). Microtitre plate bioassays for determining the MIC_{50} values were based on modified protocols outlined in the Clinical and Laboratory Standards Institute (CLSI), old National Committee for Clinical Laboratory Standards (NCCLS), document M27-A, National Committee for Clinical Laboratory Standards., Wayne, PA, USA. The compounds were first dissolved in dimethylsulfoxide (DMSO) (1 mg/mL) then further diluted 10-fold in SG (100 μg/mL, stock solution) (SG minimal medium: 6.7 mg/mL yeast nitrogen base w/o amino acids, 2% glucose). Next, 30 separate dilutions were made of each compound so that the microtitre plate wells had final concentrations of 97.5, 90, 80, 70, 60, 50, 40, 30, 20, 15, 10, 7.5, 5.0, 3.75, 2.5, 1.25, 1.0, 0.75, 0.5, 0.25, 0.125, 0.1, 0.075, 0.05, 0.0250, 0.0150, 0.0125, 0.01, 0.005 and 0.0025 μg/mL of each test compound or AmpB. The dilutions included respective volumes of stock solution and medium, plus 10 μL (approx. 5 × 10^3 cells) of stock yeast culture to bring the final volume to 200 μL/ well. Control wells included 20 μL DMSO up to 200 μL SG. All bioassay dilutions were done in triplicate. The plates were incubated at 30 °C for 48 h. Level of yeast cell reproduction for each concentration of the test compounds and AmpB was determined by optical density (OD) using a microtitre plate reader (Microplate Reader Model 680, Biorad) at 655 nm. OD readings were converted to% cell reproduction (OD concentration/OD control). The mean of the triplicate readings was then determined. MIC_{50} values were determined by probit analysis of % cell reproduction versus concentration of test compounds or ampB using the statistical analysis program provided by SPSS (Chicago, IL, USA).