SYNTHESIS AND ANTIMICROBIAL EVALUATION OF OXAZOLE-1,4-NAPHTHQUINONES

Claudia G. T. de Oliveira[a], Vitor F. Ferreira[a*], Cícero Freitas[b], Jupira M. Carballido[c]

[a]Departamento de Quimica Orgânica, Instituto de Quimica, Universidade Federal Fluminense, Campus do Valonguinho, Niteroi, 24020-150, RJ, Brazil.
[b]Departamento de Biologia Celular, Instituto de Biologia, Universidade Federal Fluminense, Campus do Valonguinho, Niteroi, 24020-150, RJ, Brazil.
[c]Hospital Universitario Antonio Pedro, Universidade Federal Fluminense, Microbiologia, Niteroi, 24030-210, RJ, Brazil.

Abstract: Several routes for preparing oxazole nucleus fused to 1,4-naphthoquinone moiety were studied. Three new oxazole-1,4-naphthoquinone derivatives (**4a-c**) were prepared and evaluated against phatogenic bacteria. The use of ortho-ester methodology was found to be the best synthetic method for preparing these oxazoles, which showed very low antibacterial activity. The intermediate **2** showed a broad spectrum of activity comparable with oxacillin and vancomycin.

Introduction

Many natural 1,4-naphthoquinones have been the subject of interest since Wendel[1] showed in 1946 that certain 2-hydroxy-3-alkyl-naphthoquinones inhibited the growth of *Plasmodium*. Afterwards several studies proved that the toxicity of naphthoquinones to *Plasmodium sp.* is due to interaction with the mitochondrial respiratory chain[2]. This observation led Fieser and collaborators to start an extensive search for new quinones[3] aiming to discover new drugs for malaria chemotherapy. These resulted in the discovery of 3-(8-cyclohexyl-octyl-2-hydroxy-1,4-naphthoquinone (menoctone), a potent inhibitor of NADH and succinate-cytochrome reductases of *Plasmodium lophurae*[4]. The antibacterial and antiprotozoan activities of 2-hydroxy-3-alkyl-1,4-naphthoquinones have been summarized by several authors.[5,6] Besides these biological activities, various other heterocyclic quinones[7] posses activities against several types of cancer cells[8] (i.e. mitomycins, etc.), virus[9,10] and fungi[11] More recently, β-lapachone, an *ortho*-pyran-naphthoquinone, was intensely investigated for clinical use in cancer chemotherapy[12].

The occurrence of the oxazole nucleus in a wide variety of natural and unnatural biologically active compounds[13], as well as the utilization of oxazoles as useful reagents has provided a continuing stimulus for the development of new compounds of this class. Several cytotoxic and antifugal macrolides containing two or three oxazole rings have been isolated from marine living organisms such as nudibranch egg masses,[14] sponges[15], and stony corals.[16] It noteworthy the following natural products, among many other, having the oxazole moiety: Halishigamides A-D[17], Calyculin C[18] and Hennoxazole A.[19] Synthetic oxazole compounds also had showed interesting biological effects such as cytotoxic[20], analgesic[21], antibacterial.[22]

As an ongoing program devoted to synthesize 1,4-naphthoquinone[23] and oxazole derivatives, we have decided to synthesize new 1,4-naphthoquinone analogues having the oxazole ring attached at 2,3-positions and test then against several phatogenic bacteria.

Experimental

Melting points were observed on a Reichert micro hotstage and are uncorrected. Analytical grade solvents were used. Column chromatography was performed on silica gel 60 (Merck 70-230 mesh). Infrared spectra were recorded on a Perkin-Elmer 783 spectrophotometer. NMR spectra were recorded on a Varian Unity Plus VXR (300 MHz) in deuteriochloroform solutions and tetramethylsilane was used as the internal standard (δ=0 ppm). Low resolution electron-impact mass spectra (12 eV) were measured in a Hewlett Packard 5985 instrument and high resolution fast atom bombardment mass spectra (HRFABMS) were recorded on a 3-NBA matrix in the positive ion mode on a VG ZAB-E mass spectrometer. Freshly purified samples were used for measuring physical constants and spectral data.

Hydroxy-3-amino-1,4-naphthoquinone (**3**)

A mixture of lawsone **1** (5.75 mmol) in HCl 5% (v/v, 7.5 mL) under stirring was dissolved in dioxane (20 mL). The solution was cooled externally with ice and solid sodium nitrite (1.16 g, 16.8 mmol) was slowly added keeping the temperature below 5 °C. The reaction was monitored by TLC until complete consumption of **1**. The reaction was allowed to warm to room temperature and extracted with dichloromethane (3 x 20 mL). The combined organic phase was extracted with cold water (3 x 15 mL) and dried over anhydrous Na_2SO_4. The solution was concentrated under reduced pressure yielding **2** (90%) as a yellow crystalline solid.

A solution of **2** in ethanol (10 mL) was warmed to 50 °C then added slowly in three portions of 5 mL a freshly prepared solution of $Na_2S_2O_4$ 10% (w/v, 15 mL). The mixture changed from yellow to deep purple and after 15 min a solid started to form. The reaction was kept undisturbed at room temperature for 24 h. The solid material was collected by vacuum filtration and air-dried giving **3** (50%) as a purple solid, m.p. 132 °C (EtOH) (lit. 130-140 °C).

General procedure for preparing **4a-b** and **6a-b** by acid chloride method

To a solution of **3** (5 mmol 0.945 mg) in xylene (100 mL) were added the appropriated acid chloride (5.5 mmol), triethylamine (5.5 mmol, 0.76 mL) and PPTS (piridinium *p*-toluenesulfonate) (1.3 mmol, 325 mg). The reaction was refluxed during 16 h. (8 h for **6a** and **6b**). The products were isolated by extraction with AcOEt (3x25 mL) and separated by silica gel column chromatography eluted with hexano/AcOEt (15%). The yields are reported in Table 1.

General procedure for preparing **4a-c** by *ortho*-ester method

A mixture of **3** (3 mmol, 567 mg) in xylene (70 mL), and the appropriated ortho-ester (3 mmol) and PPTS (0.8 mmol, 199 mg) was refluxed for 16 h. The mixture was extracted with AcOEt and washed with $NaHCO_3$ 5% (3 x 10 mL) and then evaporated. The residue was chromatographed on silica gel column eluted with hexane / AcOEt (10%).

4a from **6a**

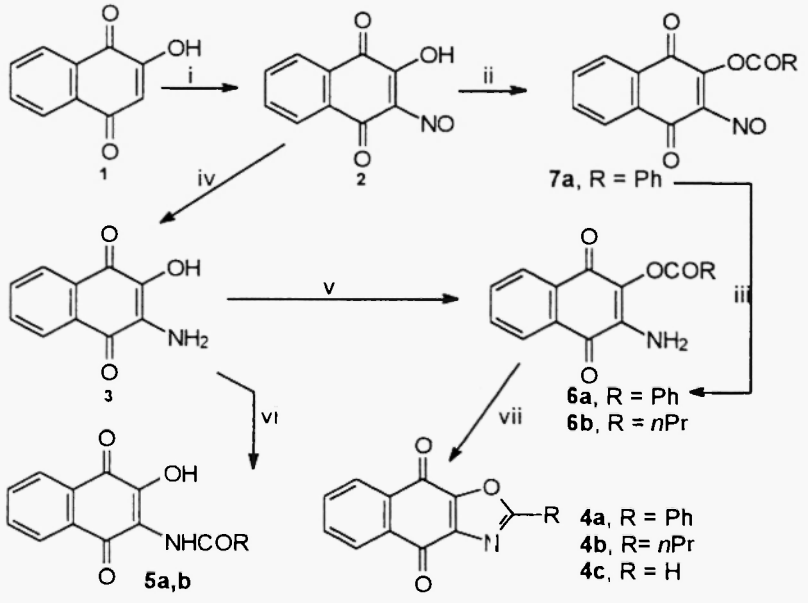
To a solution of **6a** (1 mmol, 293 mg) in xylene (20 mL) was added PPTS (0.3 mmol, 75 mg) and refluxed for 10 h. The product **4a** was isolated in 60% (165 mg) by extraction with AcOEt and purified by silica gel column chromatography and eluted with hexane/AcOEt (7:3).

2-Benzoyl-3-nitroso-1,4-naphthoquinone (**7a**)

A solution of **2** (3 mmol, 609 mg), benzoyl chloride (3.3 mmol, 0.38 mL) and triethylamine (3.3 mmol, 0.46 mL) in dichloromethane (50 mL) was stirred under nitrogen at room temperature overnight. The reaction mixture was poured into water (100 mL). The organic phase was separated, washed with $NaHCO_3$ 5% (3x15 mL) and dried over anhydrous Na_2SO_4. The solution was evaporated under reduced pressure and the residue recrystalyzed in CCl_4 producing **7a**.

2-Benzoyl-3-amino-1,4-naphthoquinone (**6a**) from **7a**

To a solution of **7a** (1 mmol, 307 mg) in methanol (50 mL) was added $NaBH_4$ (4 mmol, 152 mg) in portion wise for 30 min at room temperature. The mixture was poured into saturated solution of NH_4Cl (50 mL) and extracted with AcOEt (3 x 15 mL). The organic phase was dried over anhydrous Na_2SO_4, evaporated under reduced pressure and the residue purified by column chromatography over silica gel eluted with hexane/AcOEt (9:1) producing **6a** in 69% (202 mg) yield.



i) $NaNO_2$/HCl; ii) PhCOCl, Et_3N, CH_2Cl_2; iii) $NaBH_4$, EtOH; iv) $Na_2S_2O_4$, H_2O, v) RCOCl, PPTS, Et_3N, 8h; vi) RCOCl, PPTS, Et_3N, 16h; vii) $RC(OMe)_3$ PPTS, Xylene

Scheme 1. Synthetic sequence for preparing compounds **4a-c**, **5a-b**, **6a-b** and **7a**.

Biological Essay

Microbial cultures growth conditions. Tested microorganisms included the following Gram-positive bacteria: *Staphylococcus aureus* and *Staphylococcus epidermidis*, and for Gram-negative: *Escherichia coli*, *Klebsiella pneumoniae* and *Shigella flexneri*. All bacteria used in this study were isolated from patients at the University Hospital Antônio Pedro/UFF-RJ and grown (at 37 °C) in medium with peptone, yeast extract, sodium chloride and, dibasic-sodium phosphate. Lorian disks (7 mm diameter) were soaked in 5 $mg.mL^{-1}$ of naphthoquinones as solutions in dimethylsulfoxide (DMSO). Disks were put on an exponentially growing plated culture with appropriate dilution to $1.0x10^{7}$ colony forming unit ($CFU\ mL^{-1}$). The plates were then incubated for 24 h at 37 °C. The results were recorded by measuring the zones surrounding the disk. Control disks containing DMSO, ATCC 29.213 of S. aureus and the antibiotics oxacillin and vancomycin were used as controls in the assay. Significant results: halo > 12 mm Table 3 reports the inhibition zones (mm) of **4a-c**, **5b**, **2** and **3**.

Results and Discussion

Oxazole chemistry have been experienced a renaissance since the beginning of combinatorial methodologies Research has included the synthesis of oxazole-containing peptido-mimetics[24] and the preparation of oxazole-containing peptide macrocycles that could serve as scaffolds for combinatorial elaboration. Hence, many synthetic methodologies have appeared in the recent literature.[25]

The synthesis of oxazole-1,4-naphthoquinone derivatives **4a-c** was studied by different process (Scheme 1). The amino lawsone (**3**) is the key compound of this sequence, which was synthesized from lawsone **1** (3-hydroxy-1,4-naphthoquinone) in two steps. The first step is the conversion of **1** into nitroso-quinone **2** by nitrosation, which is then reduced with sodium dithionate in aqueous medium giving a moderate yield of 2-amino-naphthoquinone **3**.[26] Having in hands the intermediates **2** and **3**, we subsequently studied several cyclization processes to produce oxazoles derivatives.

The formation of oxazole ring from **3** was initially performed by one-pot procedure reported by Goldtein and Dambeck[27], which use an acyl chloride, triethylamine, PPTS (piridium *p*-toluenesulfonate) as catalyst in refluxing toluene. In this condition it was isolated the oxazoles **4a** and **4b** along with the amides **5a** and **5b** in low yield (entries 1 and 2). The formation of these amides suggest the ester-intermediates was formed initially but it cyclized faster than the amides. Since the scope of this reaction was not well established with amino-alcohol of 1,4-naphthquinones, and in particular, the lack of data related to steric effects as well as electronic effects, it prompted our investigation of the reaction in two separated steps. The ester-intermediates **6a** and **6b** were prepared in 68 and 72% yield (entry 3 and 4) using the same reaction conditions described above but in 8 hours. Heating the later compounds in toluene with PPTS furnished the oxazoles **4a** and **4b** in moderate to good yields.

In order to overcame the regioselectity problem (ester vs amide) in forming the amides **5a-b**, it was studied a synthetic route through nitroso-lausona **2**. Initially it was reacted with benzoyl chloride forming **7a**, which upon reduction with sodium dithionate furnished the oxazole **4a** in 60% yield (entry 7). The overall yield of this route was similar to the amino-ester in two-steps but operationally more complicated.

In a search for a direct procedure for obtaining the oxazoles we investigated the condensation of **3** with some available ortho-esters (entry 5 and 6), catalyzed by PPTS in refluxing toluene.[28] The oxazoles **4a** and **4c** were obtained in good yields. Then, it was possible to prove that in this reaction the overall yield in two-step is three-fold higher than the one-pot procedure.

Each step of these sequences is operationally convenient and reproducible. The structures of **4a-c**, **5a-b**, **6a-b** and **7a** are supported by ir, ms and NMR data based on HMBC and HMQC experiments (see Table 3).

Table 1. Summary of the yields and pathways used for preparing the compounds **4a-c**, **5a-b**, **6a-b** and **7a**.

Entry	Reagent	Route	Group	Oxazole	Amino-ester	Amide	Nitroso-ester
1	3	Direct	Phenyl	**4a** - 22%	-	**5a** - 7%	-
2	3	Direct	Propyl	**4b** - 36%	-	**5b** - 30%	-
3	3	Amino-ester	Phenyl	**4a** - 60%	**6a** - 68%	-	-
4	3	Amino-ester	Propyl	**4b** - 70%	**6b** -72%	-	-
5	3	Ortho-ester	Phenyl	**4a** - 80%	-	-	-
6	3	Ortho-ester	H	**4c** - 70%	-	-	-
7	2	Nitoso-ester	Phenyl	**4a** - 60%	-	-	**7a** - 70%

Table 2. Antimicrobial activity* of **4a-c**, **6a-b** and **7** as determined by diffusion techniques.

Oxazole	*Kreisiella pneumoniae*	*Staphlococcus epidermidis*	*Shigella flexneri*	*Staphlococus. aureus*	*Escherichia coli*
4a	12	7	5	8	7
4c	11	12	zero	18	4
4b	10	14	zero	10	zero
5b	10	11	zero	14	3
3	9	9	6	12	14
2	24	29	24	35	20

S. aureus* ATCC 29.213: oxacillin = 45 mm and vancomycin = 24 mm; **4c- 2-H-4,9-dioxo-4,9-dihydro-naphtho[2,3-*d*] oxazole, **4a**- 2-phenyl-4,9-dioxo-4,9-dihydro-naphtho[2,3-*d*] oxazole; **4b**- 2-n-propyl-4,9-dioxo-4,9-dihydro-naphtho[2,3-*d*] oxazole; **5b**- 2-hydroxy-3-N-propylamide-1,4-naphthoquinone; **3** -2-hydroxy-3-amino-1,4-naphthoquinone; **2**- 2-hydroxy-3-nitroso-1,4-naphthoquinone.

All the compounds shown in Table 2 are inhibitors of the bacteria used in this research, but only **2** (35 mm) had a broad spectrum of activity comparable with oxacillin = 45 mm and vancomycin = 24 mm (commercially available antibiotics). The oxazole derivatives **4a-c** and the intermediates **5b** showed very low antibacterial activities. All the other intermediates were inactive.

Since the only active compound has a nitroso group bonded to 1,4-naphthoquinone moiety. We can speculate that this broad spectrum of activity is due to the formation of highly reactive nitrosyl species *in vitro*[29], activated by bacterial NADH- and NADPH-dependent reductases.

Conclusions

In summary, we have studied several routes for preparing 1,4-naphthoquinones **4a-c** having the oxazole ring fused to quinone moiety. The formation of oxazole ring from **3** by one-pot procedure already reported in the literature, showed to be inadequate for producing these compounds in good yields due to regioselectivity problem. It was possible to show that this procedure in two separated steps produces the oxazoles in higher yield. The use of ortho-ester was found to be the best synthetic method for preparing **4a** and **4c**. All the compounds were tested against several types of Gran-positive and Gran-negative bacteria. The oxazoles derivative **4a-c** and most of the intermediates showed very low antibacterial activity. However, the nitroso-lawsone **2** showed a broad spectrum of activity comparable with oxacillin and vancomycin, which are commercially available antibiotics. It was expected some activity for these oxazoles derivatives, since the oxazole nucleus is presented in a wide variety of natural and unnatural biologically active. These results may provide some important information for future design of antibacterial drugs having the oxazole nucleus.

Acknowledgment

National Research Council of Brazil (CNPq) and FAPERJ supported this investigation. The individual fellowships granted to V.F.F. and C.G.T.O. by CNPq are gratefully acknowledged.

Table 3: Some Physical data of compounds **2**, **4a-c**, **5a-b**, **6a** and **7**.

	Mol	MS (m/z)	i.v ν (cm^{-1})	H^1 NMR ($CDCl_3$)	C^{13} NMR ($CDCl_3$)
2	$C_{10}H_7O_4N$ 203	M^+ 203 (20); 172 (72); 104 (100); 76 (48).	3310 (OH); 1690, 1665 (CO); 1530 (C=N)	8.25 (1H, dd, J = 7.6 and 1.1 Hz, H_5); 8.20 (1H, dd J = 7.6 and 1.1 Hz H_8); 7.93 (1H, d, J = 7.6 and 1.4 Hz, H_6); 7.84 (1H, dt, J = 7.6 and 1.4 Hz, H-).	173.1 (C_1); 159.2 (C_2); 136.0 (C_3); 183.0 (C_4); 132.3 (C_{4a}); 125.3 (C_5 and C_8); 135.0 (C_5); 132.8 (C_-); 126.1 (C_8); 130.6 (C_{8a}).
4a	$C_{17}H_{13}O_3N$ 275	$[M + H]^+$ FAB 276.0660 (Δ= 3.7)	1685, 1690 (CO); 1580 (C=N)	8.35-8.24 (4H, m, H_5, H_8 H_2, $H_{2'}$); 7.85-7.79 (2H, m, H_6 and H-) 7.63-7.53 (3H, m H_3, $H_{3'}$, $H_{5'}$).	166.1 (C_2), 143.9 (C_{3a}); 178.5 (C_4); 131.9 (C_{4a}); 128.1 (C_5 C_8); 134.2-134.1 (C_6 C_7); 132.2 (C_8); 173.1 (C_9); 150.1 (C_{9a}); 125.0 (C_1); 126.9-127.3 ($C_{2'}$, C_6); 132.8-129.0 (C_3, $C_{4'}$, $C_{5'}$).
4b	$C_{14}H_{11}O_3N$ 241	M^+ 241 (80);172 (100); 104 (95); 76 (35).	1680, 1695 (CO); 1540 (C=N)	8.20-8.13 (2H, m H_5, H_8); 7.75-7.69 (2H, m, H_6, H-) 2.91 (2H, t, J = 7.5 Hz $H_{1'}$); 1.88 (2H, sext J = 7.5 Hz, $H_{2'}$); 0.98 (3H, t, J = 7.5 Hz, H_3).	170.6 (C_2), 143.0 (C_{3a}); 178.3 (C_4);131.7 (C_{4a}); 127.2 (C_5); 134.0 (C_6); 134.2 (C_7); 126.8 (C_8); 132.3 (C_{8a}); 173.2 (C_9); 150.5 (C_{9a}); 30.3 ($C_{1'}$); 20.1($C_{2'}$), 13.5 ($C_{3'}$).
4c	$C_{13}H_9O_3N$ 199	M^+ 199 (100), 171 (85), 104 (68), 76 (48).	1660, 1650 (CO); 1550	8.28 (1H, s H_2); 8.30-8.23 (2H, m, H_5, H_8) 7.84-7.81 (2H, m, H_6, H-)	154.8 (C_2), 141.9 (C_{3a}); 177.8 (C_4); 131.7 (C_{4a}); 127.3 (C_5); 134.2 (C_6); 134.5 (C_7); 127.0 (C_8); 132.4 (C_{8a}); 173.3 (C_9); 150.2 (C_{9a}).
5a	$C_{17}H_{11}O_4N$ 293	M^+ 293 (15) 104 (80)	3320 (OH); 3030 (NH); 1660,1650 (C=O).	8.18-8.02 (4H, m Ar); 8.01-7.20 (2H, m H_5, H-); 7.75-7.62 (3H, m H_4, $H_{2'}$, $H_{5'}$); 3.49 (1H, s D_2O, OH); 9.85 (1H, s D_2O NH).	181.1 (C_1); 152.6 (C_2); 121.2 (C_3); 180.5 (C_4); 131.3 (C_{4a}); 126.0 (C_{5-8}); 134.8 (C_5); 133.5 (C_7); 130.1 (C_8); 165.4 (C_9); 133.6 ($C_{2'}$); 128.0 ($C_{3'-6'}$); 128.4 ($C_{4'/6'}$); 131.9 ($C_{5'}$).
5b	$C_{14}H_{13}O_4N$ 259	M^+ 259(10), 104 (68), 76 (48).	3290 (OH) 3030 (NH); 1690 1650, 1620 (CO)	8.12 (1H, qd J = 5.4, 3.0 0.6 Hz H_5); 7.75-7.69 (2H, m, H_6, H-); 8.08 (1H, qd J = 5.4, 3.0 0.6 Hz H_8); 2.54 (2H, t, J = 7.2 Hz, $H_{2'}$); 1.80 (2H, sext, J = 7.2 Hz $H_{3'}$) 1.04 (3H, t, J = 7.2 Hz $H_{4'}$); 8.45 (1H, s, OH); 12.95 (1H, s, NH)	180.1 (C_1); 145.5 (C_2); 119.8 (C_3); 174.6 (C_4); 130.7 (C_{4a}); 126.2 (C_5); 133.9 (C_6); 133.8 (C_7); 126.6 (C_8), 129.8 (C_{8a}); 174.7 (C_9); 38.6 ($C_{2'}$); 18.8 ($C_{3'}$); 13.3 ($C_{4'}$)
6a	$C_{17}H_{11}O_4N$ 293	M^+ 293(10), 104 (86), 76 (40).	3300 (NH_2); 1780 (CO); 1670, 1690 (CO).	8.28 - 8.25 (2H, m H_5, H_8); 8.14-8.05 (2H m, $H_{2'}$, H-); 7.69-7.90 (2H, m H_6, H-); 7.79-7.72 (3H, m, $H_{4'}$, $H_{5'}$, $H_{6'}$).	174.5 (C_1); 141.9 (C_2); 125.5 (C_3); 181.5 (C_4); 127.2 (C_{4a}); 130.2 (C_5); 135.1 (C_6); 135.0 (C_7); 128.6(C_8); 128.6 (C_{8a}); 167.4 ($C_{1'}$); 129.3 ($C_{3'}$, $C_{7'}$); 129.2 ($C_{4'}$); 128.1 ($C_{5'}$); 132.9 ($C_{6'}$).
7a	$C_{17}H_9O_5N$ 307	M^+ 307 (12), 279 (75), 256 (100).	1745 (CO), 1672, 1665 (CO); 1580 (C-NO).	8.27 (2H, dd J = 7.2 , 1.5 Hz, H_5, H_8); 8.06 (2H, dd J = 6.9 1.5 Hz $H_{3'}$ H-); 7.76 (3H, t, J = 8.1 Hz, H_4 , H_5 , $H_{6'}$); 7.93 (2H, dt, J = 7.2, 1.5Hz, H_6 H-)	167.3 (C_1); 135.2 (C_2); 172.1 (C_3); 130.4 (C_{4a}); 128.1 (C_5, C_8); 130.8 (C_6, C_7); 130.4 (C_{8a}); 162.3 ($C_{1'}$); 130.4 (C_2); 129.3 and 129.4 ($C_{3'}$, $C_{7'}$); 132.9 ($C_{4'}$, $C_{6'}$); 128.6 ($C_{5'}$).

[a] ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity Plus 300 spectrometer operating at 300 and 75 MHz respectively, with TMS as the internal standard, following experiments 1D (^{1}H, PND and DEPT, θ=90° e 135°) and 2D (^{1}H x ^{1}H- COSY, ^{1}H x ^{13}C-COSY, nJ_{CH} n =1,2 ou 3; [b]Infrared spectra were recorded on a Perkin-Elmer 1420 spectrophotometer.

References

1. W. B. Wendel. Fed. Proc. 5. 406 (1946).
2. a) E.G. Ball, C.B. Anfinsen. O. Cooper. J. Biol. Chem. **168**, 257 (1947); b) T.H. Porter, K. Folkers, Angew. Chem. Im. Ed. **13**, 559 (1974).
3. L.F. Fieser, E. Berligner, F.J. Bondhus. F.C. Chang, W.G. Dauben, M.G. Ettlinger, G. Fawaz, M. Fields, M. Fieser. C. Heildelberger, H. Heymann, A.M. Seligman, W.R. Vaughan, A.G. Wilson. E. Wilson, M. -I. Wu, M.T. Leffler. K.E. Hamilin, R.J. Hathaway. E.J. Matson. E.E. Moore. M.B. Moore. R.T. Rapala, H.E. Zaugg, J. Am. Chem. Soc. **70**. 3151 (1948) and references cited therein.
4. a) L.F. Fieser. J.P. Schirmer. S. Archer. R.R. Lorez, P.I. Pfaffenbach, J. Med. Chem. **10**, 513 (1967) and references cited therein; b) M. Fry. M. Pudney. Biochem. Pharmacol. **43**, 1545 (1992).
5. A.T. Hudson. A.W. Randall. M. Fry. C.D. Ginger. B. Hill, V.S. Latter, N. McHardy, R.B. Willian, Parasitol. **90**. 45 (1985).
6. a) A.V. Pinto. V.F. Ferreira. R S. Capella. B. Gilbert. M.C.R.F. Pinto. J.S. Silva, Trans. Roy. Soc. Trop. Med. Hyg. **81**. 609 (1987); b) V.F. Ferreira. A.V. Pinto. F.F. Filho. M.M. Kamesiro. I.S. Silva, S.C. Santos, C.N. Pinto. M.C.F.R. Pinto. B. Gilbert. Mem. Inst. Oswaldo Cruz **87**. 345 (1992); c) W. Leon, A.M. Gonçalves, M.E. Vasconcellos, R. Do Campo. F.S. Cruz. V. Souza Mol. Biochem. Parasitol. **1**. 167 (1980); d) R. Ribeiro-Rodrigues, W.G. Santos, A.B. Oliveira. V. Snieckus. C.I. Zani. A.J. Romanha. Bioorg. Med. Chem. Lett. **5**. 1509 (1995); e) P. Boehm, K. Cooper, A.T. Hudson. J.P. Elphick. N. McHardy. J. Med. Chem. **24**. 295 (1981).
7. M. Tisler. Adv. Heterocy. Chem. **45**. 37 (1989).
8. a) H. Kappus. Biochem. Pharmacol. **35**. 1 (1986); b) T.R. Tritton, G. Yee, Science **217**, 248 (1982); c) J. Seguca-Aguilar. K. Jonsson. U. Tidefelt. C. Paul. Leukemia Res.**16**. 631 (1992); d) H. Babich, A. Stern, J. Appl. Toxicol. **13**. 353 (1993); e) C.C. Cheng. Q. Dong. D.F. Luo. F. Liu. A.Y. Chen. C. Yu, N. Savaraj, T.C. Chou, J. Med. Chem. **36**, 4108 (1993).
9. a) A.V. Pinto. M.C.R.F. Pinto. M.H.C. Lagrota. M.D. Wigg, A.N.S. Aguiar. Rev. Microbiol. **14**, 21 (1983); b) H. Laatsch. Angew. Chem., Int. Ed. **33**. 422 (1994).
10. T. Kanoshima. M. Kozuka. J. Koyama. T. Okatami. J. Nat. Prod. **52**. 987 (1989).
11. T.J. Monks. R.P. Hanzlik. G.M. Cohen. D. Ross. D.G. Grahen. Toxicol. Appl. Pharmacol. **112**, 2 (1992).
12. a) J.J. Pink. S.M. Planchon. D.A. Boothman. J. Biol. Chem. **275**. 5416 (2000) and references cited therein; b) J.J. Pink. S.M. Planchon. S. Wuerzberger-Davis. S. Exp.Cell. Res. **255**. 144 (2000); c) C.J. Li, Y. -Z. Li, A.V. Pinto. A.B. Pardee. Proc. Natl. Acad. Sci. **96**. 13369 (1999); d) C.J. Li. Y.-Z. Li, A.V. Pinto. A.B. Pardee. Mol. Med. **5**, 232 (1999).
13. C. A. Maryanoff. In Heterocyclic Compounds: Turchi, I. J., Ed.; Wiley & Sons: New York, 1986; v. 45. Chapter 5
14. a) J. A Roesener. P. J. Scheuer. J. Am. Chem. Soc. **108**. 846 (1986); b) S. Matsunaga, N. Fusetani, K. Hashimoto. J. Am. Chem. Soc. **108**. 847 (1986).
15. J. Kobayashi. O. Murata. H. Shigemori. T. Sasaki. J. Nat. Prod. **56**. 787 (1993).
16. M.A. Rashid. K.R. Gustafson. J.H. Cardellina. M.R. Boyd. J. Nat. Prod. **58**. 1120 (1995).
17. J. Kobayashi. M. Tsuda. H. Fuse. T. Sasaki. Y. Mikami. J. Nat. Prod. **60**. 150 (1997).
18. P.M. Pihko. A. M. P. Koskinen. J. Org. Chem. **63**. 92 (1998)
19. T. Ichiba. W.Y. Yoshida. P.J. Scheuer. J. Am. Chem. Soc. **113**. 3173 (1991).
20. B.V. Doctorovich. E.M. Burgess. J. Lampropolus. D. Lednicer. D. Van Derveee. L.H. Zalkow, J. Med. Chem. **37**. 710 (1994).
21. R.E. Boyd. J.B. Press. C. R. Rasmussen. R.B. Raffa. E.E. Codd. C.D. Connelly. D.J. Bennett, AL. Kirifides. J.F. Gardocki. B. Reynolds. J.T. Hortenstein. A.B. Reitz. J. Med. Chem. **42**. 5064 (1999).
22. a) N.J. P. Broom. R. Cassels. H.-Y. Cheng. J.S. Elder, P. C. T. Hannan, N. Masson, P.J. O'Hanlon, A. Pope. J.M. Wilson. J. Med. Chem **39**. 3596 (1996); b) P. Brown. D.T. Davies. P.J. O'Hanlon. J.M. Wilson. J. Med. Chem. **39**. 446 (1996).
23. a) C.G.T. Oliveira. F.F. Miranda. V.F. Ferreira. C.C. Freitas. R.F. Rabello. J.M. Carballido. L.C.D. Corrêa. J. Braz. Chem. Soc. **12**. 339 (2001); b) C.E.M. Carvalho. V.F. Ferreira. A.V. Pinto. M.C.F.R. Pinto. W. Harrison. Dyes & Pigments in press (2002).
24. a) U. Grabowska. A. Rizzo. K. Farnell. M. Quibell. J. Comb. Chem. **2**. 475 (2000); b) L.M. Martin, B.-H. Hu. Tetrahedron Lett. **40**. 7951 (1999); c) B. Clapham. C. Spanka. K.D. Janda; Org. Lett. **3**. 2173 (2001).
25. a) D.A. Evans. V.J. Cee. T.E. Smith. D.M. Fitch. P.S. Cho. Angew. Chem., Int. Ed. **39**. 2533 (2000); b) M.C. Bagley. K.E. Bashford. C.L. Hesketh. C.J. Moody. J. Am. Chem. Soc. **122**. 3301 (2000); c) S.K. Chattopadhyay, J. Kempson. A. McNeil. G. Pattenden. M. Reader. D..E. Rippon. D. Waite. J. Chem. Soc., Perkin Trans. 1 **15**, 2415 (2000); e) A.B. Smith III; G.K. Friestad. J. Barbosa. E. Bertounesque. J. J.-W. Duan.; K.G. Hull, M. Iwashima. Y. Qiu, P.G. Spoors. B.A. Salvatore. J. Am. Chem. Soc. **121**. 10478 (1999); f) R.J. Mulder. C.M. Shafer. T.F. Molinski, J. Org. Chem. **64**. 4995 (1999); g) D.R. Williams. D.A. Brooks. M.A. Berliner. J. Am. Chem. Soc. **121**. 4924 (1999); M. Lautens, A. Roy, Org. Lett. **2**. 555 (2000).
26. G.E. Podrebarac. C.C. Cheng. J. Org. Chem. **35**. 281 (1970).
27. Goldstein. W. S.. Dambeck. J. P.; J. Heterocycl. Chem. **27**. 335 (1990).
28. K.R. Kunz. E.W. Taylor. H.H. Hutton. B.J. Blackburn. Org. Prep. Proced. Int. **22**. 613 (1990).
29. G.T. Miwa. R. Wang. R. Alvaro. J.S. Walsh A.Y.H. Lu. Biochem. Pharmacol. **35**. 33 (1986).

Received on February 10, 2002