Synthesis of 2-Methyl-(*Z*)-4-(phenylimino)naphth[2,3-*d*]oxazol-9-one, a Monoimine Quinone with Selective Cytotoxicity toward Cancer Cells

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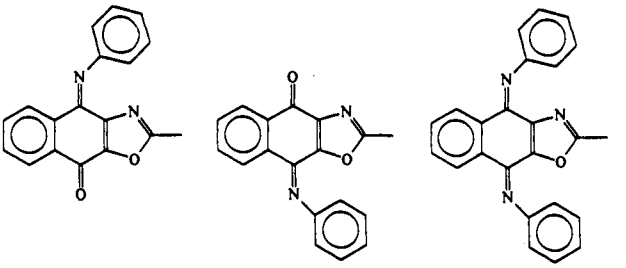
A regio and stereospecific synthesis of 2-methyl-(*Z*)-4-(phenylimino)naphth[2,3-*d*]oxazol-9-one (1) was achieved by using titanium tetrachloride in methylene chloride in the preparation of the imine. The regiochemistry was assigned by single-crystal X-ray analysis. *In vitro* tests showed that this diastereomer is selectively active for some solid cancer tumors.

Introduction

The National Cancer Institute has established an *in vitro* disease-oriented primary antitumor screen consisting of 60 human tumor cell lines.[1,2] In this *in vitro* antitumor screen, several 2-substituted naphth[2,3-*d*]oxazole-4,9-quinones demonstrated selective activity against some cancer cell lines. In this class, a mono(phenylimino) derivative of unknown regiochemistry displayed increased potency, but attempts to repeat its preparation failed.[3] We report the rational synthesis, structure determination, and selective cytotoxicity toward cancer cells of the most active of this family, the phenylimine 2-methyl-(*Z*)-4-(phenylimino)naphth[2,3-*d*]oxazol-9-one (1, NSC-650573).

Results and Discussion

Imine synthesis is achieved by dehydration resulting from removal of water or by use of drying agents. For highly hindered carbonyls, these methods give low yields. Titanium tetrachloride has been reported to be an effective Lewis acid and a water scavenger for the facile condensation of unsymmetrical ketones with amines to give the mixtures of *cis* and *trans* imines.[4,5] Exposure of the parent quinone to titanium tetrachloride and aniline in methylene chloride gave the single isomer 1, whose structure was determined by X-ray crystallography, to give the ORTEP structure (Figure 2). The regiospecificity of this reaction deserves comment. The MNDO SCF charge densities[6] (Figure 3) of the oxygen atoms in the 4- and 9-carbonyl groups are virtually the same and both should complex equally well with the titanium tetrachloride. However, the charge density at the oxazole nitrogen is over twice that at the oxygen in this heterocyclic ring. Thus, the octahedral complex 2 is more favorable than the opposite edge of the quinone and directs the condensation to this 4-position.[7] When toluene was used as solvent, the starting material was not all solubilized. The reaction went much slower, and so the selectivity was lost as three products were isolated from the reaction. One corresponded to the imine 1, another to a different monoimine (2), and the third to a diimine (3). We presume that these compounds have the phenyl groups proximate the heterocyclic ring, as products with this stereochemistry are less sterically hindered.



1 2 3

Figure 1.

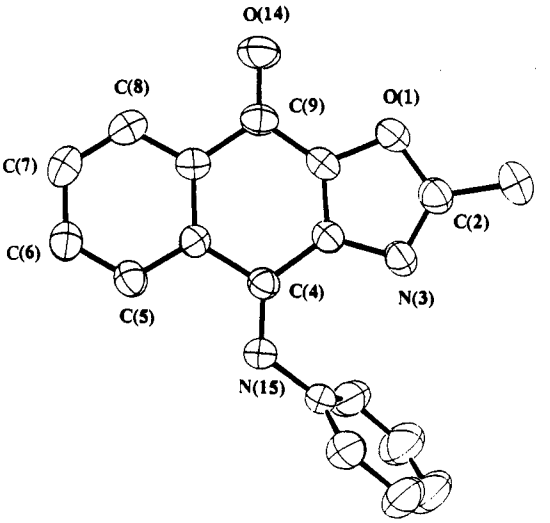


Figure 2. ORTEP view of 2-methyl-(*Z*)-4-(phenylimino)naphth-[2,3-*d*]oxazol-9-one (1).

These synthetic compounds were submitted to the NCI for testing in its *in vitro* screen. The assay is oriented to find new anticancer drugs with selectivity against a certain disease. Differential growth inhibition is analyzed for approximately 60 cell lines that belong to eight types of solid tumors and leukemia.[8,9] The assay results are reported as dose–response curves and as mean graphs[1] that facilitate the comparison of the data. Table 1 shows mean graph-medium (MG-MID) that measures the average sensitivity of the drug and the range that gives an idea of the selectivity. The best values are for compound 1. Table 2 depicts the more significant results for compound 1; most of the melanoma and breast cancer cell lines showed increased sensitivity compared to the other cell panels tested.

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Atom No. (type)	Charge
1 (O)	-0.0797
2 (C)	0.0883
3 (N)	-0.1701
4 (C)	0.318 8
5 (C)	-0.0901
6 (C)	-0.0911
7 (C)	-0.0814
8 (C)	-0.0933
9 (C)	0.3288
10 (O)	-0.2433
11 (O)	-0.2316

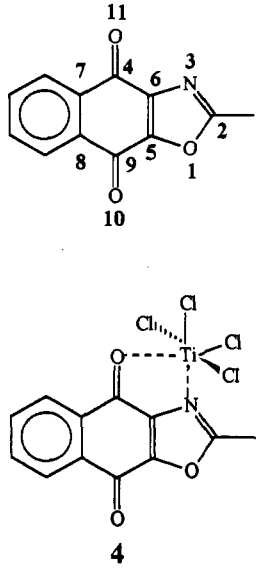

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Figure 3. MNDO-SCF charge densities for 2-methylnaphth-[2,3-*d*]oxazole-4,9-dione and its complex with titanium tetrachloride.

Table 1. *In Vitro* Tumor Cell Growth Inhibition log Concentration (M)

	TGI[a]	
compd	MG-MID[b]	range[c]
1	-5.13	2.02
2	-4.62	1.44
3	-4.02	0.49

[a] TGI is concentration which reduces cell growth to level at the start experiment. [b] MG-MID is the mean graph medium for all cell lines tested. [c] Range for the TGI values pertaining to the MG-MID.

Table 2. *In Vitro* Tumor Cell Growth Inhibition log Concentration (M)

	1	
cell line	TGI[a]	LC_{50}[b]
Melanoma		
LOXIMVI	-5.6	-5.2
MALME-3M	-5.6	-5.3
M14	-5.9	-5.4
SK-MEL-2	-5.6	-5.2
SK-MEL-5	-5.6	-5.3
Breast Cancer		
MDA-MB-435	-6.0	-5.5
MCF7	-5.3	>-4.0
MDA-MB-231/ATCC	-4.7	>-4.0
MDA-N	-6.0	-5.5
BT-549	-5.5	-5.2
T-47D	-5.1	>-4.0
HS 578T	-5.3	>-4.0

[a] TGI is concentration which reduces cell growth to level at the start experiment. [b] Concentration which reduces cell growth to 50% of level at start of experiment.

Experimental Section

General. Melting points were determined on a Koffler hot-stage apparatus equipped with a digital thermometer and are uncorrected. ^{1}H NMR spectra were recorded for deuteriochloroform solutions on a Varian Gemini-300 (300 MHz) spectrometer. All chemical shift are reported downfield (δ) relative to a tetramethylsilane internal standard. Mass spectra were recorded on a VG Analytical 70-SE mass spectrometer equipped with a 11-250J data system, and all exact mass determinations were recorded at 10 000 resolution. UV-visible spectra were recorded in a Shimadzu UV-2101PC spectrophotometer. 2-Methylnaphth-[2,3-*d*]oxazole-4,9-dione was synthesized from 2,3-dichloro-1,4-naphthoquinone as previously reported.[10] Attempts to reproduce a reported synthesis[11] were unsuccessful. 2,3-Dichloro-1,4-naphthoquinone was purchased from Aldrich Chemical Co. and used without further purification. All microanalyses were performed by Atlantic Microlab, Norcross, GA, and are within 0.4% of theoretical values.

Synthesis of 2-Methyl-(*Z*)-4-(phenylimino)naphth[2,3-*d*]-oxazol-9-one (1). Method A. To a stirred solution of 2-methylnaphth[2,3-*d*]oxazole-4,9-dione (1.5 g, 7.0 mmol) in dry CH_2Cl_2 (1.6 L) were added titanium tetrachloride (0.4 mL, 3.6 mmol) and aniline (2.2 mL, 24.2 mmol) successively. After 5 min, more titanium tetrachloride (0.15 mL, 1.4 mmol) was added, and the mixture was stirred for 10 min. Then it was filtered, and the filtrate was washed with water (2 × 1 L). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to yield a solid that was purified by flash silica column chromatography. A main fraction of a dark orange solid eluted with CH_2Cl_2–hexane (3:1) was recrystallized (CH_2Cl_2–pentane), yielding 2-methyl-(*Z*)-4-(phenylimino)naphth[2,3-*d*]oxazol-9-one (1) (980 mg, 49% yield), mp 190–192 °C (1,2-dichloroethane–hexane). ^{1}H NMR (δ, ppm): 2.47 (s, 3H), 6.97 (d, 2H, J = 7.4 Hz), 7.21 (t, 1H, J = 7.4 Hz), 7.40 (t, 2H, J = 7.4 Hz), 7.70 (t, 1H, J = 7.6 Hz), 7.77 (t, 1H, J = 7.6 Hz), 8.30 (d, 1 H, J = 7.6 Hz), 8.60 (d, 1H, J = 7.6 Hz). UV ($CHCl_3$): λ_{max} [nm] 250, 256, 292, 339, 464 (ϵ 16 140, 15 955, 22 480, 6610, 3035). EIMS: *m/e* 288 (M^+, 73), 259 (100), 231 (11), 190 (38), 77 (20). Exact mass: 288.0905 (288.0899 expected for $C_{18}H_{12}N_2O_2$). Anal. ($C_{18}H_{12}N_2O_2$) C, H, N.

Method B. A suspension of 2-methylnaphth[2,3-*d*]oxazole-4,9-dione (100 mg, 0.47 mmol) and aniline (0.16 mL, 1.76 mmol) in toluene (4 mL) was stirred at room temperature for 5 min. Titanium tetrachloride was added (0.035 mL, 0.32 mmol), and the reaction mixture was stirred for 2 h. After filtration, the filtrate was poured into water (50 mL) and extracted with chloroform (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to yield a solid. This was purified by preparative thin-layer chromatography using hexane–ethyl acetate (2:1.2) as an eluent. Fraction R_f = 0.65 provided orange crystals of (*Z,Z*)-4,9-bis(phenylimino)-2-methylnaphth[2,3-*d*]-oxazole (3) (20 mg, 12% yield), mp 187–189 °C (CH_2Cl_2–hexane). ^{1}H NMR (δ, ppm): 2.10 (s, 3H), 6.95 (d, 4H, J = 7.2 Hz), 7.14 (t, 1H, J = 7.2 Hz), 7.17 (t, 1H, J = 7.2 Hz), 7.36 (m, 4H), 7.68 (m, 2H), 8.62 (m, 2H). UV ($CHCl_3$): λ_{max} [nm] 215, 239, 304, 439 (ϵ 9790, 13 020, 24 730, 5020). EIMS: *m/e* 365 (M^+ + 2, 100), 334 (39), 293 (8), 190 (20), 149 (14), 77 (33). Exact mass: 363.1373 (363.1371 expected for $C_{24}H_{17}N_3O$). Anal. ($C_{24}H_{17}N_3O$) C, H, N. Fraction R_f = 0.53 provided dark orange crystals of 2-methyl-(*Z*)-4-(phenylimino)naphth[2,3-*d*]oxazol-9-one (1) (5 mg, 3.5% yield), with the same physical properties described above. Fraction R_f = 0.29 provided yellow-orange crystals of 2-methyl-(*Z*)-9-(phenylimino)naphth[2,3-*d*]oxazol-4-one (2) (2 mg, 1.5% yield), mp 169–173 °C (dec, CH_2Cl_2–hexane). ^{1}H NMR (δ, ppm): 2.38 (s, 3H), 6.97 (d, 2H, J = 7.4 Hz), 7.21 (t, 1H, J = 7.4 Hz), 7.40 (t, 2H, J = 7.4 Hz), 7.74 (m, 2H), 8.36 (d, 1H, J = 7.6 Hz), 8.55 (d, 1H, J = 7.6 Hz). UV ($CHCl_3$): λ_{max}[nm] 211, 224, 244, 250, 284, 336, 444 (ϵ 13 680, 13 755, 23 970, 23 320, 19 675, 5670, 2385). EIMS: *m/e* 288 (M^+, 100), 259 (73), 231 (13), 219 (24), 190 (47), 77 (40). Exact mass: 288.0899 (288.0899 expected for $C_{18}H_{12}N_2O_2$). Anal. ($C_{18}H_{12}N_2O_2$) C, H, N.

X-ray crystal structure of 1: $C_{18}H_{11}N_2O_2$, M_r = 287.20. A needle of the approximate dimensions 0.78 × 0.19 × 0.07 mm was mounted to a glass fiber along its longest axis. The crystal system was triclinic, space group $P\bar{1}$. Cell constants a = 8.634(2), b = 7.139(2), and c = 12.539(3) Å; α = 89.75(2)°; β = 89.72(2)°; γ = 65.72(2)°; U = 704.5(3) Å^3; Z = 2; μ(Mo Kα) = 0.8 cm^{-1}; F(000) = 300. Cell dimensions were determined using a SYNTEX $P2_1$ diffractometer equipped with a graphite monochromator and molybdenum source (λ = 0.710 73 Å). Data were collected on the same instrument using ω scans with 2θ varied from 4 to 50°. A total of 2485 unique reflexions were determined of which 1532

were > 2.5σ. The structure was solved using direct methods and was refined using standard techniques.[12] A total of 200 parameters were varied in the final least-squares. The refinement converged at $R = 0.047$ and $R_w = 0.054$. Residual electron density varied from 0.18 to −0.24 e/Å^3.

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Supplementary Material Available: A listing of complete crystal data, including a line drawing with the atom numbering scheme, final atomic positional parameters, atomic thermal parameters, bond distances and angles, and mean graphs of the biological evaluations performed at the NCI (12 pages). Ordering information is given on any current masthead page.

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