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Novel *N*-quinonyl amino acids and their transformation to 3-substituted *p*-isoxazinones

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Summary. Quinonyl amino acids are building blocks in the preparation of peptides which target the quinonic drug to cancer damaged area. Novel *N*-(3-chloro-1,4-dihydro-1,4-dioxonaphthalen-2-yl)-α-amino acids **1a–f** were prepared by direct substitution of 2,3-dichloro-1,4-naphthoquinone. The quinonic moiety was reduced by $NaBH_4$ to yield the corresponding hydroquinones **2a–f**, which in acidic conditions underwent internal cyclization to yield the 3,4-dihydro-2H-naphth[1,2-b]-1,4-oxazine-2-ones (six-membered azlactones) **3a–f**.

Keywords: Amino acids – Quinones – Hydroquinones – Isoxazinones – Azlactones

Introduction

Quinonic compounds represent an important class of natural (Thompson, 1997) and synthetic (Gutierrez, 1989; Powis, 1989) drugs for the treatment of human cancer. The autitumor activity stems from their ability to undergo reversible enzymatic and non enzymatic one electron reduction, leading to the formation of semiquinone radicals (Sugioka et al., 1984; Rowley and Halliwell, 1983). Under aerobic conditions, those radicals proceed through an intricate cascade of electron transfer to form hydroxyl radicals (·OH), which are literally the main reactive deleterious species to the cell (Mimnaugh et al., 1983; Goodman and Hochstein, 1977; Bates and Winterbourn, 1982; Gutteridge and Quinlan, 1985). Selective targeting of the reactive quinonyl moiety into malignant cells might be achieved by its incorporating into peptides which have specific biological receptors. Recently, Schally (Janaky et al., 1992) and his colleagues conjugated quinones to agonists and antagonists of LH-RH (Luteinizing Hormone-Releasing Hormone) *via* the ε-amino side chain of D-lysine residue. They found that the cytotoxicity of the new peptide-drug hybrides was markedly augmented *in vitro*. Consequently, the preparation and properties of quinonyl amino acids gained both chemical and

chemotherapeutic interest. They are building blocks for the design and preparation of selected peptide sequences, serving as vehicles targeting the drug to cancer damaged area. The linking of several free and blocked amino acids to a quinone moiety, and free radical generation both *via* chemical and enzymatic means, were previously described by us (Bade Shrestha-Dawadi et al., 1996; Rahimipour et al., 1996, 1998). The reaction of benzoquinones with a few free amino acids or their esters, that leads to 2,5-substitution by the amine function on the quinone nucleus, has also been reported (Alciaturi et al., 1982; Ioffe and Khavin, 1954; Foster et al., 1974; Jones and Qian, 1998). In this paper we would like to report on the preparation of several novel α-amino acids modified by a quinonyl substitution on their α-amino functional group (**1a–f**). After reduction to their hydroquinonic form (**2a–f**) a facile internal condensation occurs to yield a new class of 3-substituted *p*-isoxazinone derivatives (six-membered azlactones) (**3a–f**).

Materials and methods

IR spectra were recorded on a Nicolet 5ZDX FI-IR spectrometer. ^{1}H-NMR were run on a Bruker WP 200 SY spectrometer and mass spectra (CI in methane) were obtained on a Finnigan 4020 quadropole spectrometer. Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected. The optical and specific rotation was determined in absolute MeOH on 341 Perkin Elmer polarimeter. The correct elemental composition was proved by high resolution mass spectrometry. All reagents were of commercial quality.

N-(3-chloro-1,4-naphthoquinon-2-yl)-L-amino acids **1a–f***; general procedure*

A solution of the L-amino acid (30 mmol) in 30 ml of 1N KOH was added to a suspension of 2,3-dichloro-1,4-naphthoquinone (2.27 gr, 10 mmol) in 150 ml of methanol. The mixture was stirred for 24 h at room temperature, then acidified with 10% HCl to pH = 1–2. The solvents were evaporated under reduced pressure and the orange or red crude product was purified using silica gel column chromatography and a mixture of CH_2Cl_2 and MeOH (9:1) as eluent (see Table 1).

Six-membered azlactones **3a–f***; general procedure*

To a stirred solution of $NaBH_4$ (5 mmol) in 25 ml H_2O was added the corresponding *N*-(3-chloro-1,4-naphthoquinon-2-yl)-L-amino acid **1a–f** (2.5 mmol). The reaction mixture was stirred under N_2 at room temperature for 5–10 minutes, acidified with 10% HCl to pH = 1–2 and stirred for a further 2–3 h. The white products precipitated from the reaction mixture after cooling, and were recrystallized from a MeOH-H_2O mixture (see Table 2).

Results and discussion

Reacting 2,3-dichloro-1,4-naphthoquinone with L-α-amino acids in a methanol/water mixture at room temperature and in the presence of one equivalent of KOH resulted in the substitution of one chlorine atom and afforded the *N*-(3-chloro-1,4-naphthoquinon-2-yl)-L-amino acids **1a–f** (Scheme 1). The

Table 1. *N*-(3-chloro-1,4-naphthoquinon-2-yl) amino acids **1a–f**

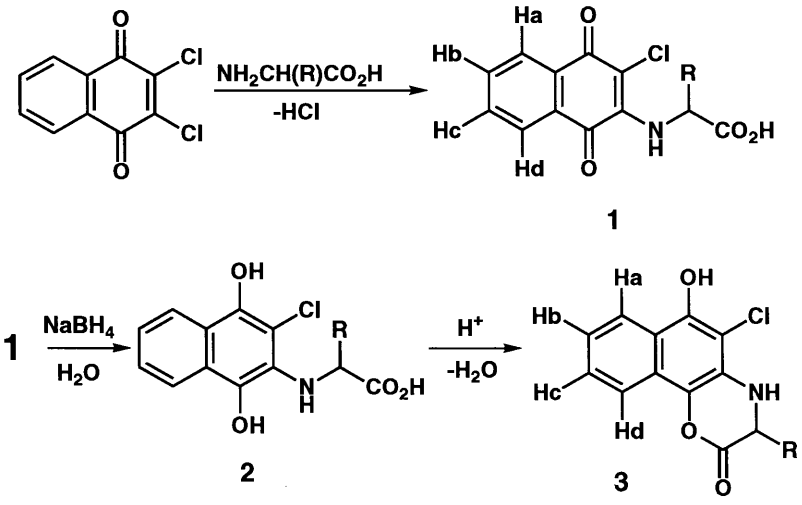
Products	Yield[b] (%)	m.p. °C	IR (KBr) ν (cm^{-1})	UV-Vis (MeOH) λ_{max} (nm) (log(ε))	^{1}H-NMR (d6-DMSO) δ (ppm) J (Hz)	MS (m/z)
1a	85	156–158	3289, 1754, 1689, 1641	218 (3.87), 238 (3.84), 278 (4.09), 474 (3.29)	13.38 (br s, 1H), 7.95 (m, 2H) 7.68–7.84 (m, 2H, 7.4, 1.6), 7.19–7.26 (m, 5H), 6.68 (br s, 1H), 5.28–5.35 (m, 1H), 3.16–3.19 (m, 2H)	355 (M^+), 342, 310, 276
1b	96	74–76	3305, 1739, 1684, 1641	228 (4.51), 278 (4.62), 478 (3.82)	9.25 (br s, 1H), 7.90–7.98 (td, 2H, 7.5, 1.3), 7.82 (td, 1H, 7.4, 1.5), 7.72 (td, 1H, 7.4, 1.6), 6.91 (d, 2H, 8.4), 6.55 (d, 2H, 8.4), 5.05 (m, 1H, 5.4), 2.97–3.11 (m, 2H)	355 (M^+-O), 325, 280, 242, 207, 172
1c	91	174–176	3422, 3308, 3207, 1700, 1681, 1655	238 (4.10), 276 (4.36), 474 (3.55)	13.18 (br s, 1H), 7.95 (dd, 2H, 7.5, 1.4), 7.56–7.87 (m, 1H, 7.3, 1.6), 7.31 (d, 1H, 7.6), 7.39 (br s, 1H), 6.90 (br s, 1H), 4.99–5.08 (m, 1H), 2.01–2.20 (m, 4H)	337 (MH^+), 208, 174
1d	80	144–145	3576, 3388, 3287, 1769, 1695, 1648	228 (4.50), 276 (4.55), 474 (3.62)	7.98 (dd, 2H, 7.3, 1.5), 7.72–7.91 (m, 2H, 7.5, 1.5), 6.87 (br s, 1H), 5.74 (br s, 1H), 5.00–5.04 (m, 1H), 3.77–3.98 (m, 2H)	296 (MH^+), 280, 251, 234, 216, 189, 172
1e	80	142–144	3465, 3323, 1730, 1683, 1636	228 (4.36), 275 (4.32), 476 (3.36)	7.98 (dd, 2H, 7.3, 1.5), 7.68–7.88 (m, 2H, 7.4, 1.6), 6.75 (br s, 1H), 5.70 (br s, 1H), 4.90 (d, 1H, 7.5), 4.30–4.37 (m, 1H), 1.20 (d, 3H, 6.4)	293 (MH^+-OH), 263, 227, 186, 172
1f[a]	81	50–52	3456, 3309, 1749, 1688, 1651	238 (4.07), 276 (4.31), 476 (3.51)	8.02 (d, 1H, 7.5), 7.91 (d, 1H, 7.1), 7.66 (td, 1H, 7.4, 1.3), 7.53 (td, 1H, 7.5, 1.5), 6.37 (br s, 1H), 5.01–5.12 (m, 1H), 2.23–2.32 (m, 1H), 1.03 (d, 3H, 6.9), 0.97 (d, 3H, 6.8)	308 (MH^+), 263, 227, 186

[a] The specific rotation measured for **1f** in MeOH is $[\alpha]_{589}^{25} = +21.1^0$. [b] Yield of isolated products based on 2,3-dichloro-1,4-naphthoquinone, not optimized.

Table 2. Six-membered azlactones **3a–f**

Products	Yield[b] (%)	m.p. °C	IR (KBr) ν (cm^{-1})	^{1}H-NMR (d6-DMSO)[c] δ (ppm) J (Hz)	MS (m/z)
3a	77	180–181	3372, 3329, 1753	9.40 (s, 1H), 7.47 (d, 1H, 8.3), 7.16 (d, 1H, 8.4), 6.89 (t, 1H, 7.6), 6.71 (t, 1H, 7.6), 6.62 (m, 5H), 5.49 (br s, 1H), 3.88 (m, 1H), 2.52 (m 2H)	340 (MH^+), 306, 214, 186, 117
3b	81	202–204	3427, 3360, 1753	9.97 (br s, 1H), 9.21 (br s, 1H), 8.05 (d, 1H, 8.4), 7.75 (d, 1H, 8.4), 7.47 (td, 1H, 7.5, 1.0), 7.29 (t, 1H, 7.5, 1.1), 6.97 (d, 2H, 8.3), 6.61 (d, 2H, 8.2), 5.94 (d, 1H, 2.3), 4.36 (m, 1H), 2.97 (m, 2H)	356 (MH^+), 330, 292, 248, 230, 180, 155, 130
3c	67	184–186	3493, 3357, 3216, 1775	9.96 (br s, 1H), 8.07 (d, 1H, 8.4), 7.78 (d, 1H, 8.5), 7.49 (td, 1H, 7.1, 1.1), 7.32 (td, 1H, 7.0, 1.1), 7.34 (br s, 1H), 6.82 (br s, 1H), 6.34 (d, 1H, 2.4), 4.16 (m, 1H), 2.32 (m, 1H), 2.19 (m, 1H), 2.04 (m, 1H), 1.96 (m, 1H)	320 (M^+), 304, 269, 242, 230, 180, 168, 130, 118
3d	70	55–57	3488, 3396 1753	9.95 (br s, 1H), 8.06 (d, 1H, 8.4), 7.76 (d, 1H, 8.4), 7.48 (td, 1H, 7.6, 0.9), 7.28 (td, 1H, 7.6, 0.9), 6.01 (br s, 1H), 5.59 (br s, 1H), 4.26 (t, 1H, 4.4), 3.79 (d, 2H, 4.5)	280 (MH^+), 251, 220
3e	75	135–137	3533, 3486, 3319, 1735	9.92 (br s, 1H), 8.05 (d, 1H, 8.4), 7.75 (d, 1H, 8.4), 7.47 (td, 1H, 7.6, 1.1), 7.27 (td, 1H, 7.6, 1.1), 5.97 (d, 1H, 2.5), 5.14 (d, 1H, 5.2), 4.03 (m, 1H), 3.96 (m, 1H), 1.16 (d, 3H, 6.0)	294 (MH^+), 276, 249
3f[a]	80	176–178	3417, 3387, 1748	9.97 (br s, 1H), 8.06 (d, 1H, 8.3), 7.77 (d, 1H, 8.4), 7.47 (t, 1H, 7.6), 7.29 (t, 1H, 7.6), 6.32 (d, 1H, 3.2), 3.89 (m, 1H), 1.92 (m, 1H), 0.90 (m, 6H)	292 (MH^+), 263, 220

[a] The specific rotation measured for **3f** in MeOH is $[\alpha]_{589}^{25} = +14.5^0$. [b] Yields of isolated products based on **1a–f**, not optimized. [c] NMR of **3a** was performed in $CDCl_3$.



1, 2, 3	R	1, 2, 3	R
a	$-CH_2C_6H_5$	d	$-CH_2OH$
b	$-CH_2C_6H_4OH$ (p)	e	$-CH(CH_3)OH$
c	$-CH_2CH_2CONH_2$	f	$-CH(CH_3)_2$

Scheme 1

involvement of equivalent amounts of the quinone and L-amino acid gave a reasonable yields. However, the use of an excess of the L-amino acid (3 equivalents) shortens the reaction time and provides higher yields of the product (up to 95%). Using this method, L-phenylalanine, L-tyrosine, L-glutamine, L-serine, L-threonine and L-leucine were linked to 2,3-dichloro-1,4-naphthoquinone. All these quinonyl L-amino acids are novel compounds.

All the new *N*-(3-chloro-1,4-naphthoquinon-2-yl)-L-amino acids (**1a–f**) were fully characterized by IR, UV-Vis, ^{1}H-NMR and mass spectrometry (see Table 1).

The molecules being unsymmetrical, the four naphthalenic protons of compounds **1a–f** show different chemical shifts in the ^{1}H-NMR spectra. Protons Ha and Hd (see Scheme 1) are almost magnetically equivalent in the range 7.90–8.02 ppm. They are splitted by *ortho* protons (Hb and Hc, respectively) and by *meta* protons (Hc and Hb, respectively). This explains why their signal appears as a double doublet (J = 7.1–7.5 Hz and 1.5–1.6 Hz). Due to the above-mentioned *meta* couplings and to the very close values of the constants $J_{Ha\text{-}Hb}$, $J_{Hb\text{-}Hc}$ and $J_{Hc\text{-}Hd}$ (J = 7.3–7.5 Hz), the protons Hb and Hc show two

overlapping double triplets and not two splitted double doublets as could have been predicted (see Fig. 1).

The NH proton appears usually as a broad peak or a doublet in the range δ = 6.37–6.90 ppm. In the IR spectra two typical quinonic carbonyl absorptions appear in the region of 1630–1690 cm^{-1}. The carboxylic carbonyl absorbs at 1700–1769 cm^{-1} and the NH is observed around 3300 cm^{-1}. In the UV-Vis the benzenoid π to π^* transition appears at 218–238 nm. One quinonic intense allowed π to π^* transition is also observed at 272–278 nm and a n to π^* transition at 468–478 nm. In the mass spectra, almost all the quinonyl amino acids **1a–f** gave the typical MH^+ parent ions. *N*-quinonyl-L-threonine (**1e**) and *N*-quinonyl-L-tyrosine (**1b**) gave the MH^+-OH and M^+-O parent peaks.

Reduction of the *N*-(3-chloro-1,4-naphthoquinon-2-yl)-L-amino acids **1a–f** to the corresponding hydroquinones was performed using $NaBH_4$ in aqueous solution and under nitrogen (performing the reaction under atmospheric conditions lowers the yield due to the fast reoxidation of the hydroquinone by

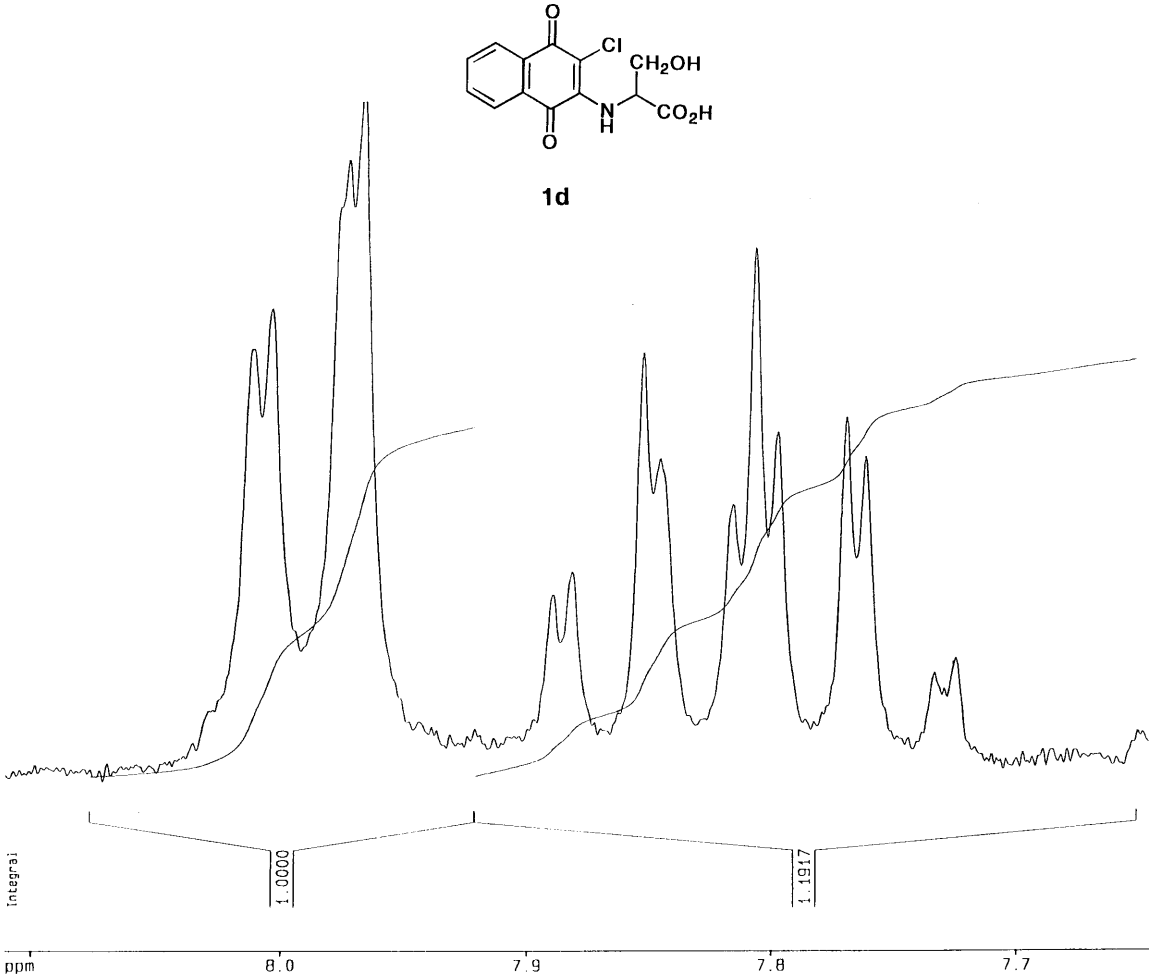


Fig. 1. ^{1}H-NMR spectrum of the aromatic region of *N*-(3-chloro-1,4-naphthoquinon-2-yl)-L-serine (**1d**)

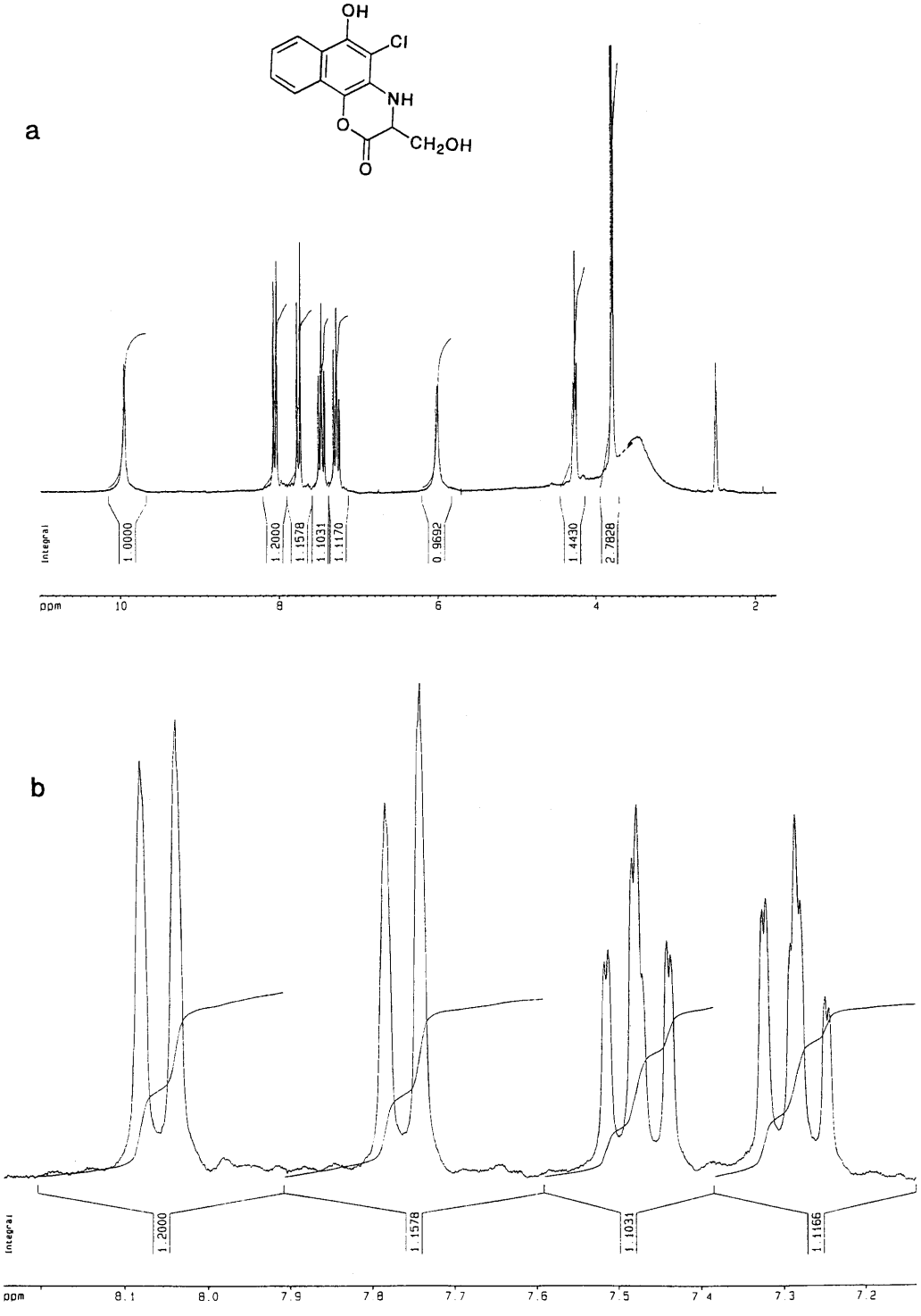


Fig. 2. **a** ^{1}H-NMR spectrum of *p*-isoxazinone **3d**; **b** expansion of the aromatic region

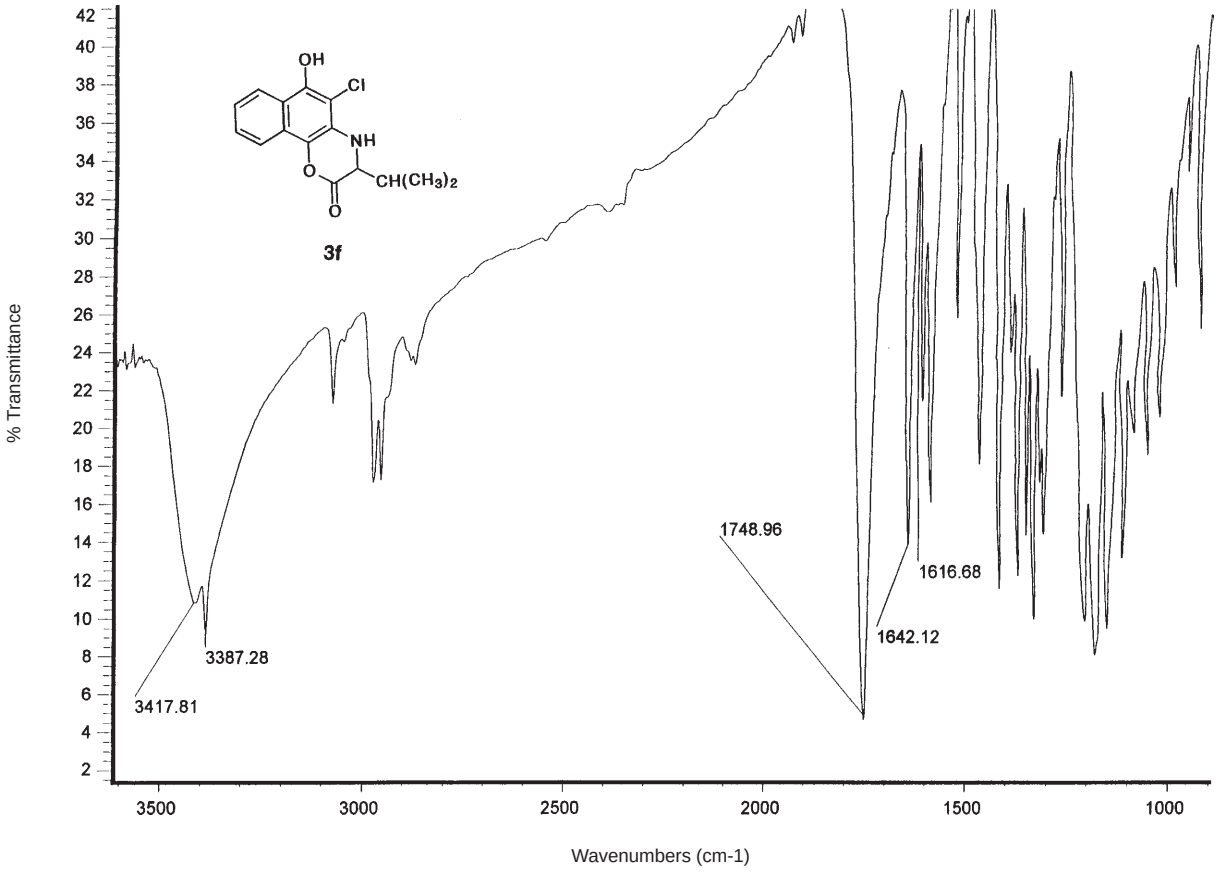


Fig. 3. IR spectrum of *p*-isoxazinone **3f**

oxygen). The reduction is very fast and, in most cases, after 5–10 minutes no quinone was detected by TLC. The products **2a–f** were pure enough for further transformation without the need of isolation. Thus, the solution was acidified with 10% HCl to pH = 1–2, which caused intramolecular esterification to give the six-membered azlactones **3a–f** in 70–80% yield (Scheme 1). In most cases the azlactones precipitated from the cooled reaction mixture, and were purified by recrystallization from a mixture of methanol-water. These compounds are very stable under unhydrous conditions. However, in the presence of moisture, the sensitive lactonic bond is slowly hydrolyzed to yield the hydroquinones **2a–f** which, in the presence of air, reoxidize to give back the starting compounds **1a–f**.

All the new azlactones **3a–f** were characterized by their IR, ^{1}H-NMR and mass spectrometric analyses (see Table 2).

The NMR signals in the aromatic region are typical of unsymmetrical naphthalenic compounds. As in **1**, all four protons have different chemical shifts. Ha and Hd resonate at δ = 7.16–8.06 ppm and Hb and Hc at δ = 7.27–7.49 ppm. The splitting pattern is very similar to that in compounds **1a–f** (see Fig. 2).

The α-CH of the lactonic ring resonate as a multiplet at the range δ = 3.8–4.2 ppm, and the NH proton appears as a doublet at δ = 5.9–6.3 ppm, which is typical of amines bonded to a conjugated aromatic system. The phenolic protons resonate around δ = 9.2–9.9 ppm. In the IR spectra of **3a–f**, the NH-absorption are observed around 3300 cm^{-1} and the OH-absoption around 3400 cm^{-1}. The lactonic carbonyl group appears at 1735–1775 cm^{-1} (see Fig. 3).

All the azlactones **3a–f** exhibit the MH^+ parent ions in the mass spectrum. The large fragments are derived from fission of the C-Cl, CH-R, COO-C and C-NH bonds.

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