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New quinone-amino acid conjugates linked *via* a vinylic spacer

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Summary. Chloranil and 2,3-dichloro-1,4-naphthoquinone have been linked to different natural and unnatural amino acids *via* a vinylic spacer. Two routes were developed for the facile preparation of these novel modified amino acids: the direct method, which can only be applied to secondary amines, and the indirect method (transamination reaction), which can be applied to any amino acid or ester.

Keywords: Amino acids – Quinones – Chloranil – 2,3-Dichloro-1,4-naphthoquinone – Transamination

Introduction

Quinonic compounds are ubiquitous in biological systems and are implicated in numerous cellular functions through involvement in mechanisms of electron and hydrogen transfer (Rich, 1982). In many processes such as oxidative phosphorylation, photosynthesis, blood coagulation and others the quinones act as electron acceptors in the electron-transport chain. Quinones are used as medicines in bacterial (Colwell and McCall, 1945; Ikeda, 1955), and fungal (Ter Horst and Felix, 1943) diseases and exhibit potent antimalarial capacity (Martin et al., 1973; Prescott, 1969). Quinones form also a large class of antitumor-agents approved for clinical use. The anthracycline glycosides such as mitomycines, mitoxantrone, streptonigrin or actinomycines are all quinonic compounds used in cancer chemotherapy (Rowley and Halliwell, 1983; Mimnauph et al., 1983; Goodman and Hochstein, 1977). The efficiency of the quinones in inhibiting cancer cell growth is believed to stem from their ability to participate in key cellular redox mechanisms with consequent generation of highly potent reactive oxygen species (ROS) (Thompson, 1997; Gutierrez, 1989; Powis, 1989).

Targeting the quinones and their cytotoxicity to afflicted cells might be achieved through conjugation to a vector having unique affinities to specific binding sites. Lately it was shown, that some human tumors are rich in receptors for peptide hormones such as the lutenizing hormone-releasing hormone (LH-RH) (Janaky et al., 1992). These peptide hormones might serve as a vehicle by which the quinones can be transferred to the cancer cells. Indeed, preliminary findings proved that quinonyl amino acids which were incorporated into a biological active peptide show cytotoxic and anticancer activity. This finding sparked interest in the synthesis and study of different quinone-amino acids conjugates (Rahimipour et al., 1996, 1998; Gorohovsky and Bittner, 2000).

In all these reports, the amino acids were directly linked to the quinonic moiety either *via* the α-amino group or *via* a side chain nucleophilic site. In this paper we would like to present a new type of quinone-amino acid conjugates in which the two moieties are separated by a vinylic bond.

Materials and methods

IR spectra were recorded on a Nicolet 5ZDX FT-IR spectrometer. ^{1}H-NMR was run on a Bruker WP 200 SY spectrometer. Mass spectra (CI in methane) were obtained on a Finnigan 4020 quadropole spectrometer and UV-Vis spectra were measured using Hewlett Packard 8452A diode array spectrophotometer. The correct elemental composition was proved by high resolution mass spectrometry, which was performed on High Resolution Magnetic Sector Mass Spectrometer VG Fison's AutoSpec. All chemicals and reagents were of analytical grade. Melting points were determined using a Thomas-Hoover capillary apparatus and are uncorrected.

Synthesis

Procedure for the direct preparation of compounds 3–5: To a solution of tetrachloro-1,4-benzoquinone (or 2,3-dichloro-1,4-naphthoquinone) (4.4 mmol) in toluene (60 mL), a mixture of acetaldehyde (0.27 g, 4.4 mmol) and the secondary amine (8.8 mmol) in toluene (20 mL) was added. The mixture was stirred at r.t. until the complete disappearance of the starting quinone (tlc) (2–5 hours). The blue colored solution was evaporated under reduced pressure and the resulting solid was purified by silica gel column chromatography using CH_2Cl_2 (ethyl acetate in the case of **4**) as the eluent. The products were recrystallized from n-hexane or petrol-ether (60–80°C).

The physical and spectroscopic properties for products **3–5** are:

Methyl 2-{methyl[(E)-2-(2,4,5-trichloro-3,6-dioxo-1,4-cyclohexadienyl)-1-ethenyl] amino} acetate (3): Blue crystals, Yield 82%, mp = 147–148°C. ^{1}H NMR($CDCl_3$) δ(ppm): 8.2(d, 1H, J = 12.9 Hz), 5.59(d, 1H, J = 11.5 Hz), 3.93(s, 2H), 3.78(s, 3H), 3.26(s, 3H). IR(KBr) ν(cm^{-1}): 3437, 2920, 1750, 1667, 1630, 1435. MS(m/z): 339[M + 2H]$^+$, 278(M-COOMe), 243, 149. UV-Vis (Toluene) λmax (nm): 274, 322, 596.

1-{[(E)-2-(2,4,5-trichloro-3,6-dioxo-1,4-cyclohexadienyl)-1-ethenyl] pyrrolidine}-2-carboxylic acid (4): Blue solid, Yield 45%, mp = 175–176°C; ^{1}H NMR(DMSO) δ(ppm): 8.48(d, 1H, J = 12.6 Hz), 5.55(d, 1H, J = 12.7 Hz), 5.49(m, 1H), 4.69(br.s, 1H), 3.32–3.33(br.s, 4H). IR(KBr) ν(cm^{-1}): 3469, 3057, 1624, 1559, 1457, 1405. MS, m/z: 351[M + 2H]$^+$, 306, 211. UV-Vis (EtOH) λmax (nm): 278, 324, 612.

Methyl-2-{methyl[(E)-2-(2-chloro-3,8-dioxo-naphthalene)-1-ethenyl]amino} acetate (5): Blue solid, Yield 78%, mp = 141–142°C; ^{1}H NMR($CDCl_3$) δ(ppm): 8.14(m, 1H); 8.08(m, 1H); 7.69(dq, 2H); 6.82(d, 1H, J = 12.8 Hz); 6.59(d, 1H, J = 14.9 Hz); 3.93(s, 2H); 3.78(s, 3H); 3.01(s, 3H). IR(KBr) ν(cm^{-1}): 2935, 1738, 1665, 1630, 1452. MS, m/z: 321[M + 2H]$^+$, 286, 230. UV-Vis (MeOH) λmax (nm): 281, 316, 649.

Procedure for the indirect preparation of compounds 6–9 (transamination): To a solution of 2,3-dichloronaphthoquinone (1.0 gr, 4.4 mmol) in toluene (60 mL), a mixture of acetaldehyde (0.19 g, 4.4 mmol) and diethyl amine (0.64 g, 8.8 mmol) in toluene (20 mL) was added. The solution was stirred at room temperature until the complete disappearance of the starting quinone (tlc) (60–90 min). The deep blue solution was evaporated under reduced pressure and the remaining crude (3-diethylaminovinyl-2-chloronaphthoquinone) was used without isolation or further purification. It was redissolved in a mixture of water/EtOH 1:3 (60 ml), and the appropriate amino acid ester hydrochloride (6.6 mmol) was added. The mixture was heated under reflux for 30–60 min, cooled, then concentrated *in vacuo* to about half of its volume. The aqueous solution was extracted with toluene (30 ml × 5). The combined organic layers dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude (and usually oily) product was purified on an alumina column using 5% methanol in CH_2Cl_2 as eluent.

Ethyl-2-{[(E)-2-(2-chloro-3,8-dioxo-naphthalene)-1-ethenyl]amino} acetate (6): Purple solid, mp = 135–136°C, yield 68%. ^{1}H NMR($CDCl_3$) δ(ppm): 8.41(dd, 1H, J = 7.9 Hz), 8.10(m, 2H), 7.66(m, 2H), 5.68(d, 1H, J = 13.4 Hz), 5.57(br.s, 1H), 4.36(q, 2H, J = 7.1 Hz), 4.03(d, 2H, J = 4.8 Hz), 1.37(t, 3H, J = 7.1 Hz). IR(KBr): ν(cm^{-1}): 3347, 2918, 1745, 1655, 1597, 1514; MS, m/z: 321[M + 2H]$^+$, 207, 191. UV-Vis (MeOH) λmax (nm): 278, 312, 604.

Methyl-2-{[(E)-2-(2-chloro-3,8-dixo-naphthalene)-1-ehtenyl]amino}-4-methylthio butanoate (7): Purple-red solid, mp = 175–176°C, yield 62%. ^{1}H NMR($CDCl_3$) δ(ppm): 8.28(dd 1H, J = 9.8 Hz), 8.08(m, 2H), 7.69(m, 2H), 5.87(d, 1H, J = 13.4 Hz), 5.68(t, 1H, J = 8.6 Hz), 4.38(m, 1H), 3.82(br.s, 6H), 2.65(t, 2H, J = 6.8 Hz), 1.29(t, 2H). IR(KBr) ν(cm^{-1}): 3422, 1742, 1675, 1592, 1435. Mass spectrum m/z: 380[M + H]$^+$, 321, 217. UV-Vis (MeOH) λmax (nm): 270, 324, 621.

Methyl 2-{[(E)-2-(2-chloro-3,8-dioxo-naphthalene)-1-ethenyl] amino}-4-methyl pentanoate (8): purple solid, mp = 158°C, yield 72%. ^{1}H NMR($CDCl_3$) δ(ppm): 8.35(dd, 1H, J = 9.9 Hz), 8.27(m, 2H), 8.00(m, 2H), 5.83(d, 1H, J = 13.3 Hz), 5.32(m, 1H), 4.32(t, 1H), 3.80(s, 3H), 1.60(br.s, 2H), 1.25–1.12(m, 7H). IR(KBr) ν(cm^{-1}): 3418, 1753, 1656, 1598. Mass spectrum (m/z): 362[M + H]$^+$, 331, 205. UV-Vis (MeOH) λmax (nm): 272, 318, 623.

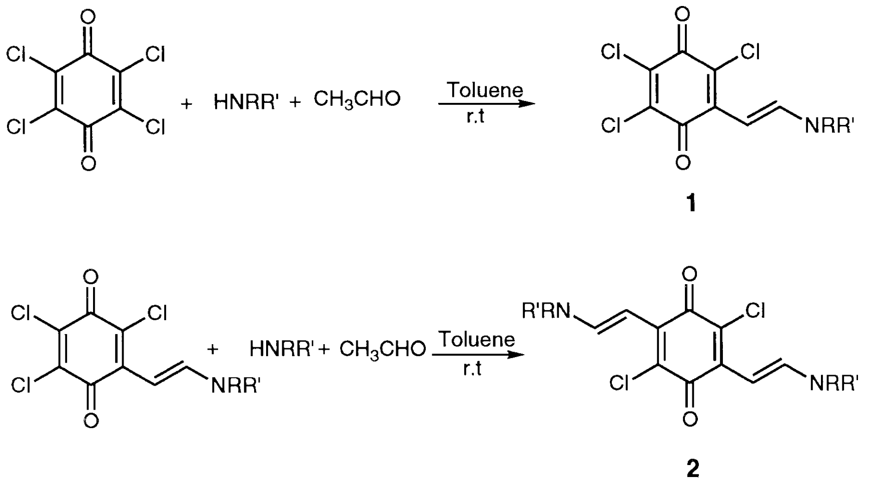
Methyl 2-{[(E)-2-(2-chloro-3,8-dioxo-naphthalene)-1-ethenyl] amino}-3-phenyl-propanoate (9): purple solid, mp = 136°C, yield 67%. ^{1}H NMR($CDCl_3$) δ(ppm): 8.27(dd, 1H, J = 9.7 Hz), 8.11(m, 1H), 7.90(q, 1H), 7.68(m, 2H), 7.31(m, 5H), 5.81(d, 1H, J = 13.4 Hz), 5.70(m, 1H), 5.42(t, 1H, J = 8.5 Hz), 4.50(d, 2H), 3.73(s, 3H). IR(KBr) ν(cm^{-1}): 3431, 1756, 1667, 1603, 1442. Mass spectrum (m/z): 398[M + 3H]$^+$, 318, 192.

Results and discussion

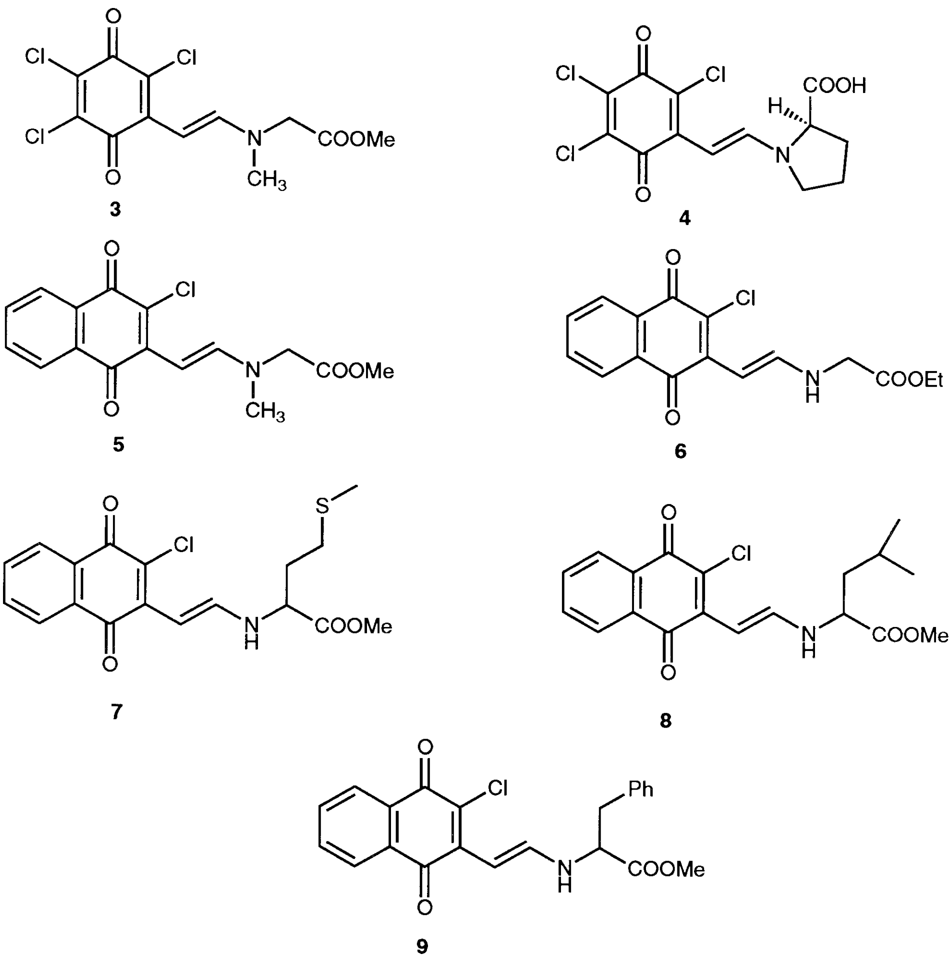
Quinonic enaminones (**1** or **2**) constitute an interesting class of quinones which can be prepared by the reaction of haloquinones with secondary amines in the presence of acetaldehyde (Alnabari and Bittner, 2000). The reaction proceeds *via* an enamine intermediate and can result in the mono-enaminone formation (the "blue" quinones **1**) depicted in Scheme 1.

As primary amines do not form enamines, this methodology could be applied only to secondary amines. Consequently this method does not fit for the preparation of quinonic enaminones of amino acids. Only amino acids containing secondary amine groups, will give the appropriate enamine and subsequently the quinonic enaminones. Indeed, the ester of the unnatural amino acid sarcosine (N-methyl glycine) and the natural amino acid proline could react directly either with chloranil or with 2,3-dichloro-1,4-naphthoquinone in the presence of acetaldehyde, to form the quinone-amino acids conjugates (**3–5**) (see Scheme 2).

Searching the literature for an alternative general method, we found that the R'RN-groups of different enaminones might be exchanged with different



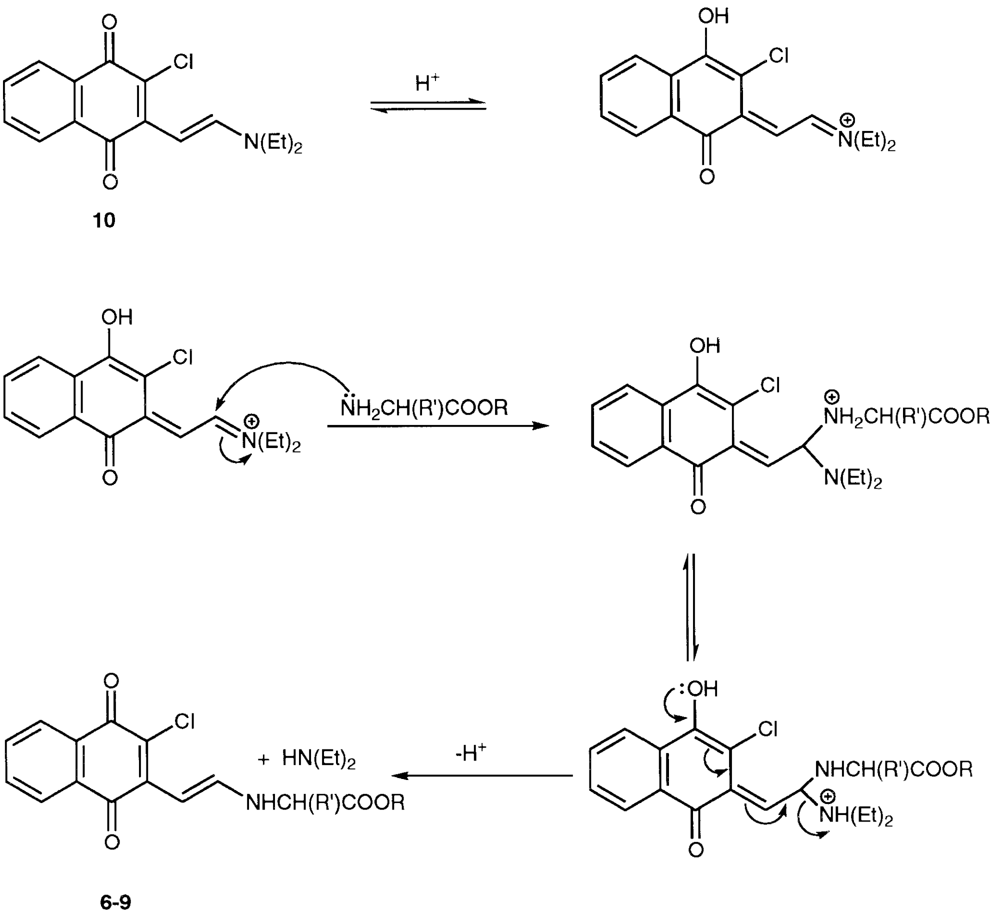
Scheme 1



Scheme 2

N- and C-nucleophiles (Svete et al., 1990; Sorsak et al., 1995; Strah et al., 1997), including primary amines. It was also reported that some quinonic enaminones are able to undergo transamination reaction with thiourea derivatives leading to the formation of heterocyclic quinones (Valters et al., 1997). Such exchange and transamination reactions offer an alternative route for the preparation of quinones-natural amino acids conjugates in which both moieties are bridged via a vinylic group. In this context, we found that the simple diethylamino naphthoquinonic enaminone (**10**) reacts with hydrochlorides of amino acids or of their esters (e.g. glycine, methionine, leucine and phenylalanine) to yield the desired "blue" conjugates (**6–9**). Acidic conditions were found to be essential to this transamination, since, under non-acidic conditions, only small quantities of the products were obtained (see Scheme 3).

Several spectroscopic features noticeable in the spectroscopic data are worth mentioning. The vinylic protons in the secondary conjugates **3–5** appear in the ^{1}H-NMR spectrum as two doublets. One at δ = 5.55, 5.59, 6.59 ppm, and



R'= H, $-CH_2CH(CH_3)_2$, $-CH_2CH_2SCH_3$, $CH_2C_6H_5$

Scheme 3

the second doublet at $\delta = 8.48$, 8.21 and 6.82 ppm respectively. The same two protons in conjugates **6–9** appear as doublets between $\delta = 5.55$–5.87 ppm and double doublets between $\delta = 8.28$–8.41 ppm. The observed coupling constants (J) are between 12–14 Hz which is indicative of an E-configuration. The full NMR data are reported in the experimental section.

The electronic absorption spectra of the "blue" naphtoquinonic enaminones compounds (**5–9**) show the expected 270–280 nm and 312–324 nm bands. In addition, another band appears between 600–650 nm. This band is known to arise from HOMO to LUMO transitions which corresponds, in this case, to an intramolecular electron transfer from the donor enamine moiety to the acceptor quinonic moiety of the molecule. In the IR spectra the two typical quinonic absorption are present in the region 1,580–1,690 cm^{-1}. The conjugated vinylic group between 2,920 and 3,030 cm^{-1}, and the NH absorption 3,300 cm^{-1} also appear. In the mass spectra the products gave the typical MH^+, $\mathrm{M} + 2\mathrm{H}^+$ or $\mathrm{M} + 3\mathrm{H}^+$ parent ions.

Two routes are now available for the facile preparation of vinyl bridged quinone-amino acids conjugates: the direct method, which can be applied to secondary amines only, and the indirect method (transamination), which can be applied to any amino acid or ester.

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