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Derivatives of aminoquinones with N-protected amino acids

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Summary

The synthesis of derivatives of aminoquinones with N-protected amino acids is reported here. 2-Amino-1,4-benzoquinone and 2-amino-1,4-naphthoquinone, prepared by the azide method in yields of 60 and 95% respectively, were coupled with N-Boc-protected amino acids including glycine, serine, proline and tyrosine, to give the correspondening derivatives. *N*, *N′*-Diisopropylcarbodiimide/1-hydroxybenzotriazole or *N*, *N′*-dicyclohexylcarbodiimide/HOBt used as coupling reagents provided the expected products in satisfactory yields and purities as supported by TLC, HPLC and spectral analysis.

Introduction

Quinones occur in nature and play an important role in certain cellular functions [1]. Covalently bound quinonoid factors have been reported to occur in many mammalian and bacterial enzymes known as quinoproteins. Many metalloproteins, all ferritins and bacterioferritins contain pyroloquinoline quinone (PPQ) or other quinonoid factors. PPQ can bind metal ions and take part in a series of protein coupled redox reactions like quinones [2,3]. Many drugs in cancer chemotherapy contain quinones [4] whereas various other uses of quinones in industry have been reported as well. Polymers formed from the reaction of quinones with amines adhere to metals with sufficient affinity to displace water from a wet, rusty steel surface, are not wettable by water and resist most organic solvents [5,6].

The synthesis of simple alkylamino derivatives of naphthoquinones and related compounds has been reported elsewhere [7]. These compounds are of considerable interest since they exhibit antitumor and antimalarial activities [8,9]. Furthermore, the synthesis of quinonyl-amino acids from 1,4 naphthoquinone or 2,3 dichloro-1,4-naphthoquinone with free or protected heterocyclic amino acids has been recently reported [10,11]. Heterocyclic amino acids play important functional and structural roles in many bioactive peptides. In addition, the aminoquinone moiety is a component of the molecular framework of several natural products, e.g. kinamycins, streptovaricins etc. [12,13].

Another approach to the synthesis of quinonyl amino acids from their amino-quinones and N-protected amino acids is the present study. Thus, we report here the synthesis of some derivatives of 2-amino-1,4-naphthoquinone and 2-amino-1,4-benzoquinone, respectively, with *N*-protected amino acids including glycine, serine, proline and tyrosine.

Materials and Methods

Quinones and N-Boc-amino acids were purchased from Sigma. All reagents and solvents used were analytical grade. Amino quinones used here were prepared as reported previously [7] with some modifications in reaction time and purification steps.

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Analysis

The purity of the compounds was established by TLC and analytical HPLC. The elution in HPL chromatography was performed under isocratic conditions using CH_3OH/H_2O 70:30 and a separon SGX C18 column (7 μm, 200 mm × 4.6 mm i.d.). TLC solvent systems used were: (1) chloroform/methanol/acetic acid 55:40:5 (v/v/v); (2) chloroform/methanol 95:5 (v/v). The products were identified by elemental analysis, UV, IR and MS spectra.

Melting points were determined on a Buchi capillary apparatus and are uncorrected. UV spectra were taken in methanol and IR in nujol or KBr discs. Mass spectra were recorded on a Hitachi-Perkin Elmer, RMU-6L spectrometer. All commercial products were used without further purification.

2-Amino-1,4-benzoquinone (**1**)

p-Benzoquinone (0.68 g, 6.29 mmol) was dissolved in 25 ml THF/H_2O (4:1) and to that solution 0.85 g NaN_3(13.07 mmol) saturated solution acidified with 3 ml glacial acetic acid was added. The resulting solution was stirred for 3 h at room temperature, the precipitate was filtered off and the solvent was concentrated in vacuo. Chromatography of the residue on a silica gel column eluting with chloroform/methanol (80:20) afforded 1. Final yield 60%, mp=148 °C, Rf_1=0.91, Rf_2=0.28, IR(nujol) 3390, 1700 cm^{-1}, UV(MeOH) 296.8, 223.6 nm, MS $[M+H]^+$ found: 124; calculated: 124.

N-Boc-glycyl-(2-amino-1,4,-benzoquinone)-amide (**1a**)

To a solution of 0.15 g (0.85 mmol) N-Boc-glycine in DMF (3 ml), equimolar amounts of DCCI (0.18 g, 0.85 mmol) and HOBt (0.12 g, 0.85 mmol) were added at 0 °C. After 20 min at that temperature, 0.21 g (1.7 mmol) 2-amino-1,4-benzoquinone was added and the mixture was stirred for 2 h at 0 °C and another 24 h at room temperature. DMF was removed under reduced pressure, the residue was dissolved in EtOAc (30 ml), washed with 1% $NaHCO_3$ (1 × 10 ml) and with brine (1 × 10 ml) very fast, dried over magnesium sulfate and concentrated in vacuo. The crude product (predominately one spot by thin layer chromatography) was subjected to analysis without further purification. Yield 53.8%, MS $[M+H]^+$ found: 281; calculated: 281.

N-Boc-seryl-(2-amino-1,4-benzoquinone) amide (**1b**)

To a solution of 0.17 g (0.83 mmol) N-Boc-serine in DMF (5 ml), equimolar amounts of DCCI (0.17 g) and HOBt (0.11 g) were added at 0 °C. After 20 min at that temperature, 0.2 g (1.66 mmol) 2-amino-1,4-benzoquinone (1.66 mmol) was added and the mixture was stirred for 2 h at 0 °C and for another 24 h at room temperature. The procedure described for 1a was followed for the recovery of the product. Yield 50%, Rf_2=0.45, MS $[M+3H]^+$ found: 313; calculated: 313.

N-Boc-prolyl-(2-amino-1,4-benzoquinone) amide (**1c**)

In analogy to the above procedures, compound 1c (overall yield 55%) was obtained as a red-brown solid which was purified on a silica gel column eluting with CH_3Cl/methanol. Rf_2=0.57, MS $[M+H]^+$ found: 321; calculated: 321.

N-Boc-tyrosyl-(2-amino-1,4-benzoquinone) amide (**1d**)

This compound was prepared as described for 1c and purified on a silica gel column with the same solvents. Yield 55%, Rf_2=0.57, MS $[M+2H]^+$ found: 388; calculated: 388.

2-Amino-1,4-naphthoquinone (**2**)

1,4-Naphthoquinone (1 g, 6.32 mmol) was dissolved in 25 ml THF/H_2O (4:1) and to that solution 1.25 g NaN_3(19.23 mmol) saturated solution, acidified with glacial acetic acid, was added. The mixture was stirred for 6 h at room temperature and upon evaporation of the solvent product 2 was obtained as a red-brown solid. Yield 95%, mp=194 °C, Rf_2=0.36, IR(nujol) 3400, 1705 cm^{-1}, UV(MeOH) 245, 210.6 nm, MS $[M+H]^+$ found: 174; calculated: 174.

N-Boc-glycyl-(2-amino-1,4-naphthoquinone)-amide (**2a**)

N-Boc-glycine (0.15 g 0.85 mmol) was dissolved in DMF (2.5 ml) and to that solution equimolar amount of HOBt and DIC were added at 0 °C. The resulting solution was stirred for 20 min at 0 °C and for another 15 min at room temperature. After filtration of the precipitate, 0.29 g (1.70 mmol) 2-amino-1,4-naphthoquinone was added to the solution and the mixture was stirred for 2 h at room temperature. The solvent was concentrated in vacuo and the remaining oil was crystallized from diethyl ether, Rf_1=0.46, MS $[M+H]^+$ found: 331; calculated: 331.

Analysis: calculated for $C_{17}H_{18}N_2O_5$(M.W. 330): C, 61.82; H, 5.45; N, 8.48. Found: C, 61.70; H, 5.43; N, 8.51.

N-Boc-seryl-(2-amino-1,4-naphthoquinone)-amide **(2b)**

N-Boc-serine (0.2 g 0.98 mmol) was dissolved in DMF (2.5 ml) and to that solution equimolar amounts of HOBt and DIC were added at 0 °C. The mixture was left for 20 min at 0 °C and for 15 min at room temperature. The precipitate was filtered off and to that solution 0.34 g (1.96 mmol) 2-amino-1,4-naphthoquinone was added and the mixture was stirred for 2 h at room temperature. The solvent was concentrated in vacuo and the residue (yield 39.8%) was purified by HPLC for MS and elemental analysis. Rf_1=0.5, MS $[M+3H]^+$ found: 363; calculated: 363. Analysis: calculated for $C_{18}H_{20}N_2O_6$ (M.W. 360): C, 60.0; H, 5.55; N, 7.77. Found: C, 60.0; H, 5.53; N, 7.89.

N-Boc-prolyl-(2-amino-1,4-naphthoquinone) amide **(2c)**

This compound was prepared as reported for 2a and 2b. The product was purified on a silica gel column eluting with chloroform/methanol. Yield 54.6%, Rf_2=0.62, MS $[M+H]^+$ found: 371; calculated: 371. Analysis: calculated for $C_{20}H_{22}N_2O_5$ (M.W. 370): C, 64.86; H, 5.94; N, 7.56. Found: C, 64.77; H, 5.81; N, 7.43.

N-CBz-tyrosyl-(2-amino-1,4-naphthoquinone) amide **(2d)**

In analogy to the above procedure for 2c, compound 2d (overall yield 37%) was obtained as a red-brown solid. Rf_2=0.3, MS $[M+H]^+$ found: 471; calculated: 471.

Results and Discussion

The preparation of 2-alkylaminonaphthoquinones in high yields via their corresponding 2-bromo-analogues has been reported [7].

Here, the two aminoquinones (2-amino benzoquinone and 2-amino-1,4-naphthoquinone) were synthesized via the azide method. Benzoquinone reacted with NaN_3 at room temperature for 3 h to give the corresponding 2-amino-1,4-benzoquinone **1** (Figure 1). During the reaction of benzoquinone with NaN_3, we observed the formation of a red to brownish precipitate, most probably of a polymeric compound (unidentified) as can be judged by TLC (R_f=0) in systems 1 and 2. The yield of this polymer was found to be 20–22% and 40% after 3 and 6 h of reaction, respectively. Amino benzoquinone was purified on a silica gel column using chloroform/methanol (90:10) and methanol, after the filtration of the first precipitate.

2-Amino-1,4-naphthoquinone **2** (Figure 1) was prepared by the same procedure as for **1**. The yield of this reaction was 95% and no polymer was formed, at least in detectable amounts by TLC, after 6 h of reaction at room temperature.

These two aminoquinones were further used for coupling with some N-protected amino acids, including: glycine, serine, proline and tyrosine. Coupling reactions were carried out at 0 °C with DIC and HOBt as activating agent in DMF in case of 2-amino-1,4-naphthoquinone derivatives and DCCI/HOBt in DMF for 2-amino-1,4-benzoquinone derivatives, respectively. In all cases the course of the reaction was followed by TLC. UV, IR and mass spectra further supported the structure of the quinonyl amino acids. Hydrolysis of the products with 6 N HCl at 110 °C for 24 h yielded the corresponding amino acid and the hydroxyquinone, as can be seen from TLC in systems 1 and 2 and from the UV absorbance of the hydrolysate (λ_{max} at 222.4 and 297.8 nm) which is characteristic of hydroxyquinone. Mass spectra were recorded on samples purified either with silica gel column chromatography or by HPLC. In most cases the protonated molecular ion $[M+H]^+$ was obtained as a high intensity peak. Interesting enough, compounds 1b and 2b gave the tri-protonated molecular ion $[M+3H]^+$ peak, whereas compound 1d gave the di-protonated one $[M+2H]^+$.

Even though quinonylamino acids can also be prepared from the corresponding quinones and free amino acids [10,11], this new synthetic approach appears to be the most promising, as aminoquinones can serve as substrates for anchoring not only amino acids but peptides of various length as well. The coupling of some di- and tripeptides with these two aminoquinones is in progress and will be reported soon. The amino-naphthoquinone moiety has been used as synthetic key intermediate for the synthesis of several biologically active compounds [14,15]. In addition, our research is focused now on the preparation of peptidoquinones, to study their hydrolysis against various enzymes and possibly upon anchoring biological active peptides, to

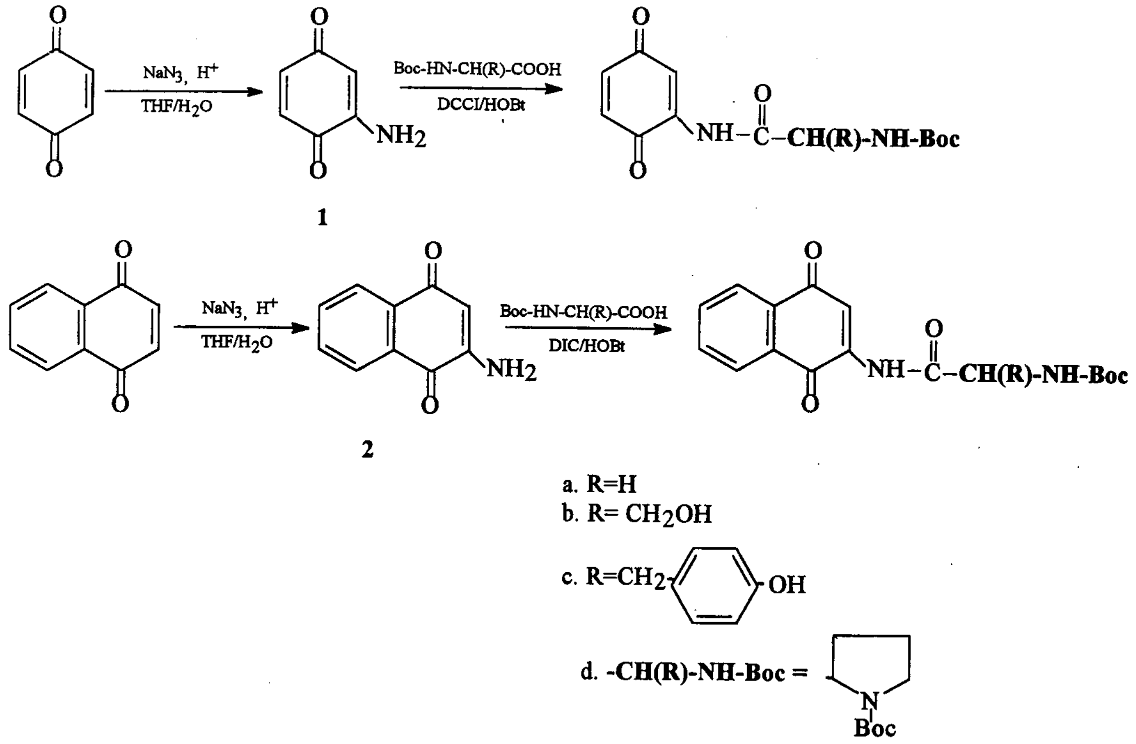

Figure 1. Derivatives of 2-amino-1,4-benzoquinone **1** and 2-amino-1,4-naphthoquinone **2** with N-Boc-amino acids; (a) glycine; (b) serine; (c) tyrosine; (d) proline.

investigate whether the biological effect of the peptide attached to aminoquinone is enhanced.

Conclusions

We have shown a new synthetic route to quinonyl amino acids from aminoquinones and *N*-protected amino acids. The reactivity of the two aminoquinones used (2-amino-1,4-benzoquinone **1** and 2-amino-1,4-naphthoquinone **2**) towards the amino acids and the coupling reagents was also studied. Naphthoquinonyl-amino acids were prepared in good yields with DIC/HOBt as coupling reagents, whereas DCCI/HOBt was found to be better for the derivatives obtained from 2-amino-1,4-benzoquinone. These new compounds might be used as substrates for new enzymes, whereas anchoring biologically active peptides to these aminoquinones might give products of significant therapeutic value.

References

1. Thomson, R.H., Naturally Occurring Quinones, 2nd ed., Academic press,: New York, NY, 1971.
2. Janes, S.M., Mu, D., Wemmer, D., Smith, A. J., Kaur, S., Maltby, D., Burlingame and Kliman, J. P., Science, 248 (1990) 982.
3. Al-Massad, F. K., Kadir, F.H.A. and Moore, G.R., Biochem. J., 283 (1992) 177.
4. Blum, R.H. and Carter, S.K., Ann. Int. Med., 80 (1974) 249.
5. Nithianandam, V.S. and Erhan S., Polymer, 32 (1971) 1146.
6. Kaleem, K., Chertok, F. and Erhan, S., Prog. Org. Coating, 15 (1987) 63.
7. Couladouros, E.A., Plyta, Z.F. and Papageorgiou, V.P., J. Org. Chem., 61 (1996) 3031.
8. Lin, T.S., Xu, S.P., Zhu, L.Y., Cosby, L. and Sartorelli, A., J. Med. Chem., 32 (1989) 1467.
9. Lin, T.S., Xu, S.P., Zhu, L.Y., Divo, A. and Sartorelli, A., J. Med. Chem., 34 (1991) 1634.
10. Bade Shrestha-Dawadi, P., Bittner, S., Fridkin, M. and Rahimpour, S., Synthesis, 12 (1996) 1468.
11. Rahimpour, S., Weiner, L., Fridkin, M., Bade Shrestha-Dawadi, P. and Bittner, S., Lett. Pept. Sci., 3 (1996) 263.
12. Furusaki, A. and Watanabe, T., Chem Pharm. Bull., 21 (1973) 931 and references therein.
13. Lanz, T., Tropf, A., Marner, F.-J., Schroder, J. and Schroder, G., J. Biol. Chem., 266 (1991) 9971.
14. Parker, K. and Sworin, M., J. Org. Chem., 46 (1981) 3218.
15. Aristoff, P. and Johnson, P., J. Org. Chem., 57 (1992) 6234.