Chinese Chemical Letters Vol. 11, No. 8, pp. 667–670, 2000

Synthesis of Some Novel 2,6- Dimethoxyhydroquinone-3-mercaptoacetyl-peptide Conjugates as Potential Antitumor Agents

Yu Fei SONG[1], Pin YANG[1]*, Leung SHEH[2]

[1] Institute of Molecular Science, Shanxi University,Taiyuan 030006
[2] Department of Chemistry,Tunghai University, Taichung, Taiwan 407-04

Abstract: This paper reports an ongoing study of the use of short chain peptides as carriers of a potential antitumor agents: 2,6-dimethoxyhydroquinone-3-mercaptoacetic acid (DMQ-MA). Three new peptide-DMQ-MA conjugates: DMQ-MA-arg-arg-ome, DMQ-MA-lys(cbz)-arg-ome, DMQ-MA-lys(cbz)-arg-arg-ome were synthesized by coupling protected amino acids in solution and the next conjugation was achieved by reacting with pentafluorophenyl ester of DMQ-MA in DMF, and further study on their ability to inhibit human pulmonary adenocarcinoma cell line (PC-9 cells) and oral epidermoid carcinoma cell line (KB) are investigating.

Keywords: 2,6-Dimethoxyhydroquinone-3-mercaptoacetic acid.

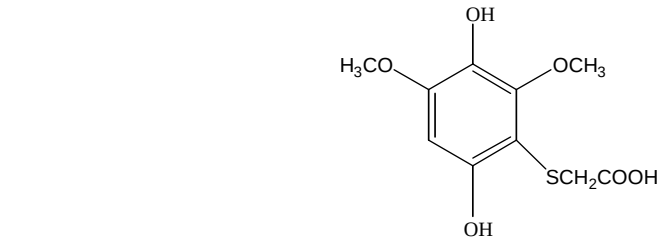
2,6-Dimethoxyhydroquinone-3-mercaptoacetic acid (DMQ-MA)[1-3] is a synthetic derivative of 2,6-dimethoxy-*p*-benzoquinone (DMQ)[4], which is a natural fermented product of wheat germ and was found to have a wide spectrum of cytotoxicity against various tumor cell lines under the synergistic activation of L-ascorbic acid as reported by G.A.Szent[5]. Owing to the very low aqueous solubility of DMQ, which is an apparent disadvantage for the development as a vaccine, we prepared the thioglycolic acid derivatives. DMQ-MA conjugates were synthesized by liquid-phase synthetic methods according to **Scheme 1** (Pfp:pentafluorophenol, DCC:N,N'-Dicyclohexyl-carbodiimide), and many of them displayed enhanced *in vitro* antitumor activities as compared to the parent compound DMQ-MA. All the previous results have showed this adequately[6].

Our preference for this study is based on the following rationale:

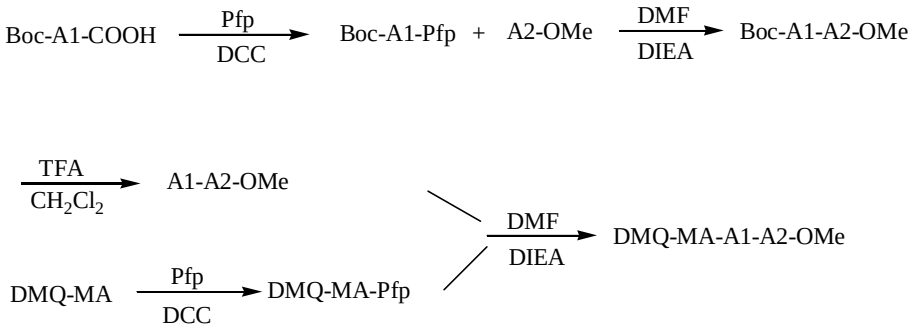
In recent years, considerable attention have been paid to drug targeting using high molecular weight carriers and some of them are successful[7]. However this method is often limited due to the high toxicity of the high molecular weight carriers to host cells[8].

Some reports showed that differences exist between cancer cells and normal cells. E.M.Ambrose[9] pointed out that the charge on the kidney surface of the mouse cancer cells is twice than that on the normal cells. J.N.Mehrishi[10] reported the change of charge density on the surface of cancer cells can inhibit the growth of the cancer cells. So we can make use of the arginine which takes positive charge under the physiological condition pH=7 so that the above goal can be met with. Arginine and lysine also may improve the solubility of the DMQ-MA conjugates.

Scheme 1.



DMQ-MA



Material and methods

All of the protected amino acid derivatives were purchased from Sigma Chemical Co. Medium pressure column chromatography was performed using Merck 230-400 mesh silica gel. TLC system was performed on Merck silica gel 60 on aluminum sheets. Low resolution mass spectra was taken from JEOL JMX-HX 110 instrument operating in the FAB mode at Tunghai University, Taiwan. Elemental analysis were performed at Cheng-Kung University, Taiwan.

Synthesis

DMQ was purchased from Aldrich, and DMQ-MA was synthesized in Prof Sheh's laboratory according to his US patent.

Boc-arg-arg-ome **1a:** N_{α}–t-Boc-L-arginine hydrochloride (0.5g, 1.61mmol) in CH_2Cl_2 was stirred with Pfp (0.44g, 2.41mmol) in ice bath for 10 min and then DCC (0.33g, 1.61mmol) was added, stirred further for 15 min with ice bath, and then stirred at room temperature for 4 hours, filtered and dried under vacuum for half an hour. Arg-ome (0.42g, 1.61mmol) in DMF then was added, treated pH=7.5 with DIEA (N,N-Diisopropylethylamine), reacted for 2-3 hours at room temperature, distilled under reduced pressure and evaporated to give a solid, which was purified by column chromatography and eluted with CH_2Cl_2, 1%, 2%, 3%, 4%, 5%, 6%, 7%,CH_3OH in CH_2Cl_2,(100ml) respectively, evaporated to get a white solid and TLC system (20%

CH_3OH in CH_2Cl_2) Rf=0.46.

DMQ-MA-arg-arg-ome **1b: 1a** in CH_2Cl_2 was stirred with trifluoroacetic acid (TFA) for 50 min and the solvents removed in vacuum. DMQ-MA (0.84g, 3.22mmol) in CH_2Cl_2 was stirred with Pfp (0.89g, 4.83mmol) in ice bath for 10 min and then DCC (0.67g, 3.22mmol) was added, stirred further for 15 min with ice bath, and then continued stirring at room temperature for 4 hours, filtered and dried under vaccum for half an hour. Then the TFA salt in CH_2Cl_2 was added, DIEA was added to adjust the pH to neutral. After 60 min the reaction mixture was filtered, diluted with CH_2Cl_2 and washed successively with citric acid , saturated $NaHCO_3$ and saturated NaCl, after which it was dried and evaporated to give a crude solid. Silica gel chromatography using stepwise elution: CH_2Cl_2, 1%, 2%, 3%, 4%, 5% CH_3OH in CH_2Cl_2, afforded the pink product **1b.** Yields:75%. TLC system (20% CH_3OH in CH_2Cl_2) Rf=0.56. Elemental analysis, Analysis calcd for $C_{23}H_{38}N_8O_8S$: C:47.10, H:6.48, N:19.11, Found : C:47.12, H:6.45, N:19.15. LRMass(FAB): $[MH^+]$:587.

2a,Boc-lys(CBZ)-arg-ome, **2b**,DMQ-MA-lys(CBZ)-arg-ome, **3a**,Boc-lys(CBZ)-arg-arg-ome, **3b**,DMQ-MA-lys(CBZ)-arg-arg-ome was prepared following a procedure similar to that for **1a** and **1b**. The results are as follows, **2b:**Yields:78%. TLC system (20% CH_3OH in CH_2Cl_2) Rf=0.51. Elemental analysis, Analysis calcd for $C_{31}H_{44}N_6O_{10}S$: C:53.75, H:6.36, N:12.14, Found : C:53.70, H:6.39, N:12.10. LRMass(FAB): $[MH^+]$:693. **3b:** Yields:70%. TLC system (20% CH_3OH in CH_2Cl_2) Rf=0.47. Elemental analysis, Analysis calcd for $C_{37}H_{56}N_{10}O_{11}S$: C:52.36, H:6.60, N:16.51, Found : C:52.38, H:6.54, N:16.55. LRMass(FAB): $[MH^+]$:849.

Results and discussion

In this experiment, there were two difficulties: 1.DMQ-MA conjugates were synthesized by reacting the dipeptides or tripeptide with the pentafluorophenyl ester of DMQ-MA in DMF. Purification problems resulting from several side products that occurred during the coupling reaction were also coming. TLC showed that there were 4 or 5 side reaction products with this method before column chromatography. So we must deal carefully with the subsequent purification with TLC monitoring the reaction products. 2.In the synthesis of DMQ-MA-peptide conjugates, the procedure is difficult because the 2,6-dimethoxyhydroquinone-3-mercaptoacetyl moiety is susceptible to minute amounts of water in the reaction medium and gradually decomposed by the Michael retrograde reaction *in situ*. Thus the reaction time was limited to 1.5-2.0h to minimize DMQ-MA's decomposition.

Based on many previous works, we are trying our best in going on the anti-cancer drug design. In this paper, arg and lys were used as carriers to bring the cytotoxic agent DMQ-MA to the target tumor molecular. In fact, we had got results[11] of the IC_{50} of DMQ-MA-arg-ome, DMQ-MA-gly-arg-ome, DMQ-MA-Ile-arg-ome as 1.08 μ M, 0.70 μ M, 3.94 μ M.(IC_{50}: *In vitro* cytotoxicity of drug concentration required to inhibit 50% of tumor cell growth). Many of these DMQ-MA conjugates displayed enhanced *in vitro* antitumor activities as compared to the parent compound DMQ-MA[1-3]. So we envisage that the three new DMQ-MA conjugates that we had synthesized can enhance the water

solubility, cytotoxicity and their inhibition effect against PC-9, KB *etc* tumor cells are now being investigated.

Acknowledgments

The authors sincerely thank Dr L.Sheh for providing chemical product, instrument and Lab for my joint research on this project in Taiwan, and acknowledg the support of the National Natural Science Foundation of China and Provincial Natural Foundation of Shanxi.

References

1. L.Sheh, *US Patent*, **1990**, *4*, 978, 783.
2. L.Sheh, C.J.Li, H.Y.Dai, V.Cheng, C.D.Chiang, N.K.Shen, C.Y.Yu, *Anti-ancer Drug Design*, **1992**, *7*, 315.
3. L.Sheh, H.Y.Dai, Y.H.Kuan, C.J.Li, C.D.Chiang, V.Cheng, *Anti-Cancer Drug Design*, **1993a,** *8*, 237.
4. D.J.Cosgrove, J.B.Hutchison, D.G.H.Daniels, T.Morgan, E.N.Greer, J.K.Whitehead, *Nature*, **1952**, *169*, 966.
5. R.Pethig, J.A.Mclaghlin, G.A.Szent, *Proc.Natl.Acad.Sci.* **1983**, U.S.A, *80*, 129.
6. W.F.Chen, M.C.Jong, S.T.Chen, L.Sheh, *Journal of the Chinese Chemical Society*, **1999**, *46*, 127.
7. L.J.Arnold, *Methods in Enzymology*, **1985**, New York, *112*, 270.
8. L.J.Arnold, J.Gutheil, N.O.Kaplan, *Proceedings of the National Academy of Science*, **1979**, U.S.A, *76*, 3246.
9. E.J.Ambrose, A.M.James, J.H.B.Lowick, *Nature*, **1956,** *177*, 476.
10. J.N.Mehrishi, *Nature*, **1970**, *278*, 364.
11. M.C.Jong, *Master thesis*, **1994**, Tunghai University.

Received 26 January 2000