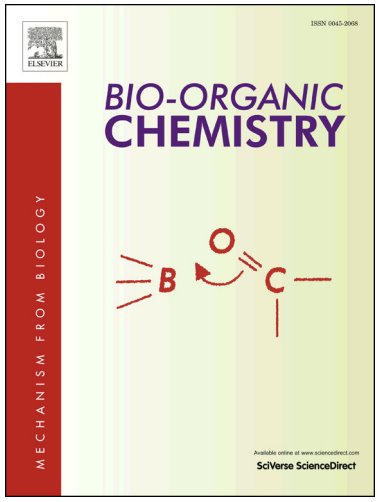


Efficient synthesis and antioxidant activities of N-heterocyclyl substituted Coenzyme Q analogues

Jin Wang, Fei Xia, Wen-Bin Jin, Jin-Yan Guan, Hang Zhao

PII: S0045-2068(16)30104-3
DOI: http://dx.doi.org/10.1016/j.bioorg.2016.08.008
Reference: YBIOO 1942

To appear in: *Bioorganic Chemistry*

Received Date: 2 July 2016
Revised Date: 7 August 2016
Accepted Date: 17 August 2016

Please cite this article as: J. Wang, F. Xia, W-B. Jin, J-Y. Guan, H. Zhao, Efficient synthesis and antioxidant activities of N-heterocyclyl substituted Coenzyme Q analogues, *Bioorganic Chemistry* (2016), doi: http://dx.doi.org/10.1016/j.bioorg.2016.08.008

Efficient synthesis and antioxidant activities of N-heterocyclyl substituted Coenzyme Q analogues

Jin Wang[a*], Fei Xia[a], Wen-Bin Jin[a], Jin-Yan Guan[a], Hang Zhao[a]

[a]*School of Pharmacy, Yancheng Teachers University, Yancheng 224007, Jiangsu Province , P. R. China*

e-mail: woongching@hotmail.com or wangj01@yctu.edu.cn

ABSTRACT:

A new strategy for the efficient synthesis of C-5 heterocyclyl substituted Coenzyme Q analogues was developed by N-alkylation of bromomethylated quinone **11** with a series of amines **12** under metal-free conditions. In vitro antioxidant activities of these Coenzyme Q analogues were evaluated and compared with commercial antioxidant Coenzyme Q_{10} by employing DPPH assay. All these N-heterocyclyl substituted Coenzyme Q analogues are found to be exhibiting good antioxidant properties and may be used as potent antioxidants for combating oxidative stress .

Keywords: CoQ analogues N-alkylation DPPH assay antioxidant activities

1. Introduction

Oxidative stress plays critical roles in the pathogenic mechanisms of several neurodegenerative disorders including Alzheimer's disease, the increased levels of free radicals and reactive oxygen species (ROS) would cause damage to cell

membrane, lipids, proteins and DNA.[1] Thus, much research effort has focused on antioxidants as potential treatment agents for oxidative stress-related diseases.[2] Coenzyme Qn (CoQ, or ubiquinone, **Fig. 1**) is a molecule with a side-chain of different hydrophobic isoprenoid units, occur naturally in all cells, acting as electron carriers in mitochondrial respiratory chain.[1,2] CoQ_{10} (n=10, **Fig.1**), the main homologue of CoQ existing in humans, is our only lipid-soluble antioxidant synthesized endogenously and efficiently prevents proteins, lipids and DNA from oxidation of reactive oxygen species (ROS). CoQ_{10} and its analogue, is widely used in the treatment of mitochondrial disorders and neurodegenerative movement disorders, such as cardiovascular disease, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, etc.[3-5] CoQ_{10} is also used as a diet supplement in elderly people to maintain health, and there is an increasing market demand.[6]

CoQ analogues with mimic of alkyl chain by other functional groups have been widely reported and also studied with biological activity, [7-13]such as inhibition of mitochondrial complex I and blood platelet aggregation, as novel inhibitors of glycation and anticancer agents, as drugs for the treatment of Alzheimer's and Parkinson's diseases, etc. But there were no antioxidant activity data reported for all these CoQ analogues. In 1989, Yoshizawa reported that some heterocyclyl-substituted CoQ derivatives could be used as drugs, such as compound **I** (**Fig.1**) which showed good inhibition of blood platelet aggregation and peroxy lipids in rat brain [13]. To the best of our knowledge, there are few methods disclosed for the preparation of these N-heterocyclyl substituted CoQ analogues, among the reported approaches, some

used dangerous or toxic reagent (n-BuLi, Ag_2O, $AgNO_3$, $Pd(PPh_3)_4$, etc), harsh reaction conditions, or multiple steps.[9-11,13] These drawbacks limited their wide application in industry and academia. Therefore, development of an efficient and environmentally friendly methods to synthesis of substituted CoQ analogues bearing a N-heterocyclic unit is desirable. Inspired by the reports that halogenmethyl-1,4-benzoquione had reactivity in an electron-transfer C-alkylation reaction,[14-16] herein, we reported a direct approach to N-heterocyclyl substituted CoQ analogues by N-alkylation of bromomethylated quinone **11** with various N-heterocyclic compounds via S_N2 reaction, and we also investigated the antioxidant activities of these synthesized CoQ analogues by DPPH assay in vitro.

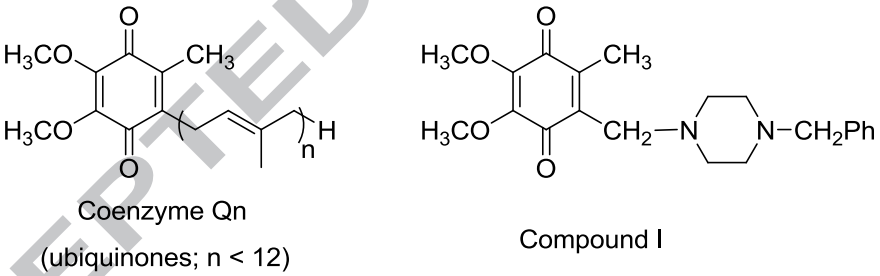


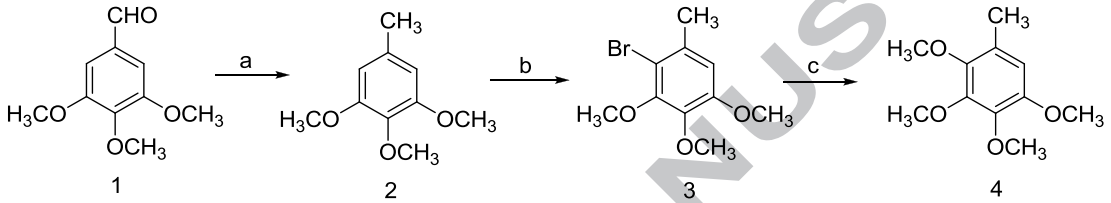
Figure 1. Coenzyme Qn and Compound I

2. Results and Discussion

2.1 Chemistry

In our previously work,[17] we described an efficient approach for the preparation of 2,3,4,5-Tetramethoxytoluene **4**. Initially, treatment of commercially available 3,4,5-trimethoxybenzadehyde **1** under Wolff–Kishner conditions provided **2** in quantitative yield, then bromination of **2** utilizing NaBr–H_2O_2 system gave bromide **3**

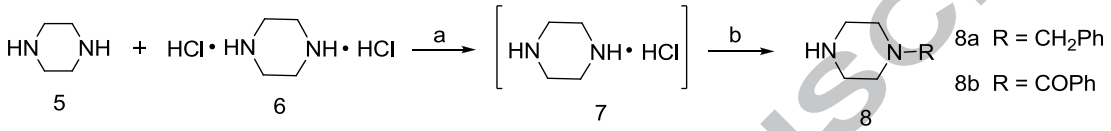
in 98% yield, finally, S_NAr reaction of **3** with CH_3ONa–CH_3OH under cuprous salts catalyst to afford **4** in overall 91 % yield (based on **1**). In view of the difficulties associated with the preparation of previous CoQ analogues, the availability of compound **4** prompted us to devise a practical route to compound **I,** an N-heterocyclyl substituted CoQ analogue which could be developed to drug.



Scheme 1. Reagents and conditions: (a) 80% $NH_2NH_2{\cdot}H_2O$, ethylene glycol, KOH,70℃/2 h, 150℃/2 h, 100 %; (b) NaBr, 30% H_2O_2, HOAc, 40℃, 1h, 98 %; (c) CH_3ONa, CH_3OH, CuCl, DMF, 100℃, 4h, 93 %

In order to synthesize compound **I**, a regioselective protected piperirazine would to be synthesized as the secondary amine for the optimization of the alkylation reaction. So we have to synthesize N-Benzylpiperazine first. There have been few reports of the direct transformation of piperazine to mono benzyl piperazine, most of them employ the direct benzylation of piperazine or start from mono-protected N-acyl- and N-aroylpiperazines under very cautious control of the pH of the reaction mixture, however, these methods always produce a mixture of dibenzylpiperazine from which N-benzylpiperazine **8a** is difficult to isolate.[18] Inspired by literature,[19] N-benzylpiperazine **8a** was achieved in 95 % yield without the need of using a monoprotected piperazine. Firstly, converting anhydrous piperazine **5** to its somewhat

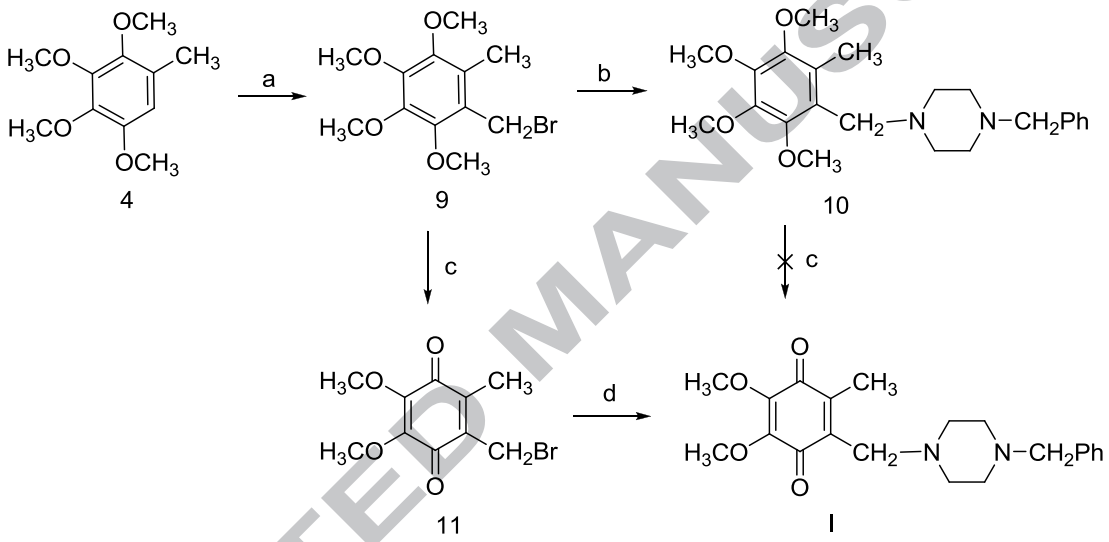
intermediate monohydrochloride **7** in situ by reaction with piperazine dihydrochloride **6** in ethanol at reflux for 2 h, following reacted with benzyl chloride at the same temperature, as shown in **Scheme 2**. Using the similar way, we also got N-benzoylpiperazine **8b** in 94 % yield.



Scheme 2. Reagents and conditions: (a) ethanol, 75℃, 2h; (b) benzyl chloride or benzoyl chloride, 75℃, 2h

Inspired by our previous work,[17e,f] we use 47 % HBr solution as Blanc Reaction reagent instead of 37% HCl. Benzylbromide **9** was obtained in 98 % yield by bromomethylation of **4** using paraformaldehyde and 47 % HBr solution under solvent-free conditions. We envision that compound **I** could be synthesized by oxidation of compound **10**, as anticipated, compound **10** was obtained in 89 % yield by amination of **9** and **8a** in the presence of K_2CO_3 in DMF at 80 ℃ for 2 h. Unfortunately, the final oxidation to compound **I**, using ceric ammonium nitrate (CAN), gave only a trace amount of the ubiquione **I** and we could not improve this yield. We speculated that the piperazine group in compound **10** might also be oxidized by CAN during the reaction, the lack of success with this step forced us to consideration of avoiding the final oxidation altogether by using bromomethylated quinone **11** directly, instead of benzylic bromide **9** (**Scheme 3**). Then we tried another

route: Firstly, oxidation of benzylic bromide **9** with CAN successfully afforded the desired bromomethylated quinone **11**,[20] following by N-alkylation of **11** (1 equiv.) with **8a** (1.1 equiv.) in the presence of K_2CO_3 (1.2 equiv.) in dichloromethane (DCM) at 25 ℃, we were delighted to know that our desired was indeed formed in 58 % yield (**Table 1**, entry 1).

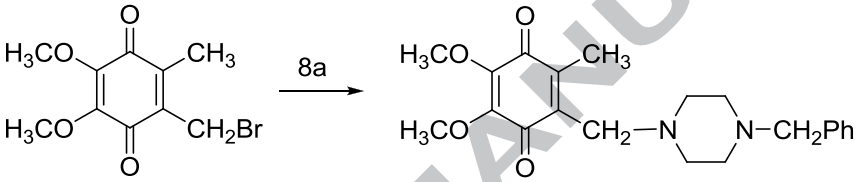


Scheme 3. Reagents and conditions: (a) (HCHO)n, 47 % HBr, 40℃, 1h, 98 %; (b) K_2CO_3, DMF, 80 ℃, 2h, 89 %; (c) Ceric ammonium nitrate (CAN), THF/H_2O, 0 ℃, 80%; (d) K_2CO_3, DCM, 25 ℃, 58 %

The N-alkylation of bromomethylated quinone **11** was investigated under different conditions, the results are summarized in **Table 1**. The yield of compound **I** was decreased from 58 % to 35 % when 10% NaOH solution was instead with K_2CO_3 (**Table 1**, entry 2), when we added 10% NaOH solution to the reaction, we found that the color of reaction mixture changed to abnormal purple which indicated the bromomethylated quinone **11** was destroyed by the strong base. Different solvents

(THF, DMF, EtOH) were also investigated, the reaction only proceed at higher temperature (60-80 ℃, **Table 1**, entries 5,7,9) not at 25 ℃ (**Table 1**, entries 4,6,8). However, lower yields were obtained in all cases (**Table 1**, entries 5,7,9), indicating that DCM is the suitable solvent and K_2CO_3 is a good base for this reaction. On basis of these screening studies, the optimal condition was using **11** (1 equiv.), **8a** (1.1 equiv.) and K_2CO_3 (1.2 equiv.) in dichloromethane (DCM) at 40 ℃ for 0.5 h.

Table 1. Table optimization of N-alkylation conditions [a]



11 I

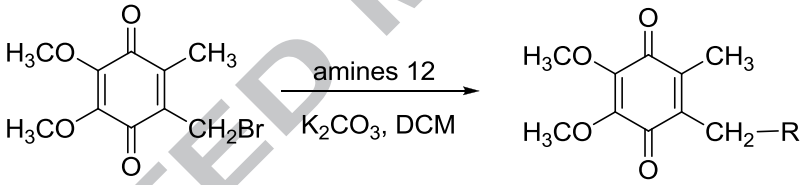
Entry	solvent	base	Time (h)	Temp (℃)	Yield [b] (%)
1	DCM	K_2CO_3	6	25	58
2	DCM	10% NaOH	6	25	35
3	**DCM**	**K_2CO_3**	**0.5**	**40**	**75**
4	THF	K_2CO_3	6	25	0
5	THF	K_2CO_3	2	60	60
6	DMF	K_2CO_3	6	25	0
7	DMF	K_2CO_3	2	80	55
8	EtOH	K_2CO_3	6	25	0
9	EtOH	K_2CO_3	2	70	62

[a] reaction conditions: **11** (1mmol), **8a** (1.1 equiv), base (1.2 equiv), and solvent (5 ml)

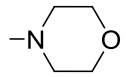
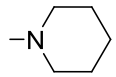
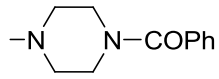
[b] isolated yields

With the optimized conditions, various commercially available amines **12** were investigated in the N-alkylation and the results were summarized in **Table 2**. The N-alkylation proceeded smoothly in the presence of K_2CO_3 in DCM at 40 ℃, commercially available amines (morpholine **12a**, piperidine **12b)** and N-benzoylpiperazine **8b** all underwent efficient amination (**Table 2**, entries 1-4). Compared with our early study,[17f] it's interesting to note that utilizing the -CH_2Br synthetic intermediate (**11**), rather than -CH_2Cl, good yields of target compounds were obtained in the N-alkylation reaction.

Table 2. N-alkylation of bromomethylated quinone **11** with different amines [a]



11 13

entry	amines **12**	R	CoQ **13**	Yield[b] (%)
1	**12a**		**13a**	70
2	**12b**		**13b**	60
3	**8b**		**13c**	80

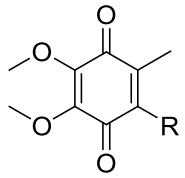
[a] reaction conditions: **11** (10 mmol), **8a** (1.1 equiv), K_2CO_3 (1.2 equiv), and DCM (10 ml), 40 ℃, 0.5 h. [b] isolated yields

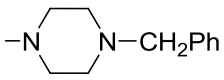
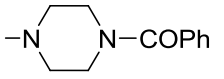
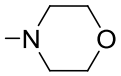
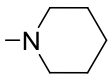
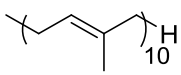
2.2 DPPH free radical antioxidant activity

DPPH radical scavenging activity evaluation is a rapid and convenient technique

for screening the antioxidant activities of the antioxidants.[26] The antioxidant activity was defined as the amount of CoQ analogues necessary to decrease the initial DPPH concentration by 50% and expressed as IC_{50}. The results of CoQ analogues (**CoQ_0, I, 13c, 13a, 13b, CoQ_{10}**) were shown in **Table 3**. Based on IC_{50} values, their DPPH radical-scavenging activity follows the order: **13c** > **I** > **CoQ_{10}** > **13a** > **13b** > **CoQ_0**. Among the compounds tested, all the compounds (**I, 13c, 13a, 13b**) showed better radical scavenging activities than Coenzyme Q_0, with IC_{50} values of 0.609, 0.294, 2.638, 3.471 mM, respectively. In addition, compound **I** and **13c** displayed better DPPH radical scavenging activity than Coenzyme $Q_{10,}$ with IC_{50} values of 1.212 mM. On the basis of the above observation, the introduce of heterocyclic substituent at the C-5 position of Coenzyme Q might increase its antioxidant activity.

Table 3. Antioxidant activities of N-heterocyclyl substituted CoQ analogues



compounds	R	IC_{50}(mM)	SD(mM)
CoQ_0	H	3.664	0.025
I		0.609	0.032
13c		0.294	0.041
13a		2.638	0.027
13b		3.471	0.039
CoQ_{10}		1.212	0.058

[a] SD, standard deviation of three experiments.

3. Conclusions

In summary, we have developed a simple, mild, efficient, and eco-friendly method for the synthesis of C-5 N-heterocyclyl substituted CoQ analogues. Moreover, we demonstrated an easy and efficient way of direct transformation of free piperazine to mono benzyl or benzoyl piperazine under metal-free conditons. All these reactions are operationally simple, environmentally friendly, easy to work-up, high yields and take place under mild conditions. Therefore, this strategy would have potential industrial application in the preparation of a wide variety of biologically active Coenzyme Q analogues.

What's more, all the N-heterocyclyl substituted Coenzyme Q analogues were evaluated for the DPPH radical antioxidant activity, experimental results demonstrated that all the synthesized CoQ analogues showed good antioxidant properties. Especially, compounds **I** and **13**c bearing a piperazine group showed better radical scavenging activities than Coenzyme Q_{10} in DPPH assay, which means compounds **I** and **13**c may displayed more potential antioxidant activities than Coenzyme Q_{10} and may be developed as the potential therapeutic antioxidants for oxidative stress-related diseases.

4. Experimental Section

General methods: The synthesised CoQ analogues were purified by silica gel (80–120 mesh) column chromatography (Adamas-beta, China) and identified by thin-layer chromatography (TLC), MS, and NMR analysis. ^{1}H NMR spectra and ^{13}C NMR were

recorded on a Bruker DRX NMR spectrometer and a API STAR Pulsar mass spectrometer, respectively. A UV-2550 UV–Vis spectrophotometer from Shimadzu was used in the scavenging assays. 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH), Coenzyme $Q_{10,}$ Coenzyme $Q_{0,}$ Ceric ammonium nitrate (CAN), piperazine, piperidine and morpholine were purchased from Adamas-beta, China. Other chemicals used were of analytical grade.

4.1 Synthesis

General procedure for the synthesis of compounds (**8a** ,**8b**).

A solution of anhydrous piperazine (**5,** 8.6 g, 0.1 mol) and piperazine dihydrochloride (**6,** 31.8g 0.2mol) in ethanol (80 mL) were heated with vigorous stirring at 75 °C for 2 h. Then a solution benzyl chloride (13.9g, 0.11mol) or benzoyl chloride (15.4g, 0.11mol) was added dropwise over a period of 40 minutes to the hot solution. The reaction mixture was refluxed for another 2 h, the progress of the reaction was monitored by TLC**.** The mixture was cooled and the precipitated piperazine dihydrochloride **6** was collected and washed three times with ethanol. The filtrate combined with the washes was concentrated *in vacuo* to give the N-benzylpiperazine or N-benzoylpiperazine hydrochloride which was then treated with 6M NaOH to pH >12. The aqueous layer of crude N-benzylpiperazine or N-benzoylpiperazine was extracted into CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated *in vacuo*. The crude oily product was purified by flash column chromatography on silica gel (MeOH-CH_2Cl_2 1:3) to give **8a** (18.4 g, 95%) or

8b (17.8 g, 94 %).

Data for **8a:**[21] ^{1}H NMR (500MHz, $CDCl_3$):□□ 7.34-7.28 (m, 5H, Ph), 3.57 (s, 2H, OBn), 3.23 (s, 4H, piperazine-H), 2.77 (s, 4H, piperazine-H). MS (ESI): *m/z* =177 $[M+H]^+$. Spectroscopic data are according to the literature. [21]

Data for **8b:**[22] ^{1}H NMR(500MHz, $CDCl_3$): 7.40 (s, 5H, Ph-H), 3.78 (s, 2H), 3.42 (s, 2H), 2.40-3.10 (m, 5H). MS (ESI): *m/z* =191 $[M+H]^+$. Spectroscopic data are according to the literature. [22]

Synthesis of compound **9** [23]

To a stirred mixture of **4** (2.12 g, 0.01mol) and paraformaldehyde (0.60 g, 0.02mol) was added 47% HBr (10ml) dropwise at room temperature. Then the mixture was stirred at 40℃ for 1 h, Water were added and the mixture was extracted with petroleum ether, and the combined organic layers were washed with brine. The solution are dried over anhydrous sodium sulfate and solvent was removed in vacuo to afford an orange oil **9** (2.98 g, 98% yield). ^{1}H NMR (400MHz, $CDCl_3$): 4.68 (s, 2H, CH_2Br), 3.93 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 2.28 (s, 3H, CH_3). Spectroscopic data are according to the literature. [23]

Synthesis of compound **10**

Compounds **9** (1.15 g, 3.8 mmol), N-benzoylpiperazine **8a** (0.67 g, 3.8 mmol) , and K_2CO_3 (0.63 g, 4.56 mmol) in DMF (10 ml) were heated at 80 ℃ for 2 h, After completion of the reaction, the crude was extracted with three portions of CH_2Cl_2.

The yellow extracts were washed with brine until neutrality, then dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude oily product was purified by a silica-gel column chromatography (PE/EtOAc 3:1) to give a yellow oil **10** (3.7 g, 89%).

^{1}H NMR (300MHz, $CDCl_3$):7.30-7.29 (m, 5H, Ph), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.49 (s, 2H, CH_2), 3.46 (s, 2H, CH_2), 2.49(brs, 8H, piperazine), 2.24 (s, 3H, CH_3); ^{13}C NMR(75MHz, MeOD): 148.9, 147.7, 146.0, 144.3, 138.1, 129.3(2C), 128.2(2C), 127.4, 127.0, 125.4, 63.1, 61.2, 61.0, 60.6, 53.3, 52.8, 11.8; MS(ESI): *m/z* = 401 $[M+H]^+$. Calcd for $C_{21}H_{27}O_4N_2$: 401.2440. Found: 401.2433.

Synthesis of compound **11** [24]

A solution of CAN (21.9 g, 0.04 mol) in water (15 mL) was added dropwise to a solution of compound **9** (3.04 g, 0.01 mol) in THF (10 mL) at 0 ℃. The mixture was stirred at room temperature for another 2 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the crude was extracted with three portions of CH_2Cl_2 (15 mL). The orange extracts were washed with brine until neutrality, then dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product were purified by a silica-gel column chromatography (PE/EtOAc 4:1) to give an orange oil **11 (**2.2 g, 80 % yield).

^{1}H NMR (500MHz, C_5D_5N-d_5): 4.29 (s，2H, CH_2Br), 3.88 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 1.99 (s, 3H, CH_3); ^{13}C NMR (125 MHz, MeOD): 183.7 (C=O), 181.6 (C=O),

144.7, 144.2, 142.3, 136.7, 61.2 (OCH_3), 61.1 (OCH_3), 35.0 (CH_2Br), 11.8 (CH_3). Spectroscopic data are according to the literature. [24]

General procedure for the synthesis of compound **I** and **13**

A mixture of compounds **11** (2.74 g, 10 mmol), amine **12** (11 mmol), and K_2CO_3 (1.66 g, 12 mmol) in CH_2Cl_2 (10 ml) were heated at 40 ℃ for 0.5 h. The progress of the reaction was monitored by TLC, after completion of the reaction, the reaction mixture was extracted with three portions of CH_2Cl_2. The orange extracts were washed with brine until neutrality, then dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Then the residue was were purified by a silica-gel column chromatography (PE/EtOAc 3:1) to give the desired compounds.

Data for compound **I :** ^{1}HNMR (500MHz, MeOD): 7.60-7.58 (m, 2H, Ph), 7.52-7.50 (m,3H, Ph), 4.46 (s, 2H, CH_2), 4.20 (s, 2H, CH_2), 4.01 (s, 3H, OCH_3), 4.00(s, 3H, OCH_3), 3.58(brs, 8H, Piperazine-CH_2), 2.20 (s, 3H, CH_3).

HRMS(ESI): *m/z* = 371 $[M+H]^+$. Calcd for $C_{21}H_{27}O_4N_2$: 371.1970. Found: 371.1969.

Data for **13c** : ^{1}H NMR (500MHz, C_5D_5N-d_5): 7.35–7.33 (m, 5H, Ph), 3.95 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.69 (brs, 2H, CH_2N), 3.37 (s, 2H, CH_2), 3.32 (brs, 2H, CH_2N), 2.49 (brs, 2H, CH_2N), 2.33 (brs, 2H, CH_2N), 2.07 (s, 3H, CH_3).

^{13}C NMR (125 MHz, MeOD): 184.4 (C=O), 183.8 (C=O), 170.2 (C=O), 144.3, 142.9, 137.3, 135.6, 129.6, 128.4, 126.9, 61.2 (2×OCH_3), 53.4 (CH_2N), 52.9 (CH_2N), 51.5 (CH_2), 47.6 (CH_2N), 42.0 (CH_2N), 12.4 (CH_3).

MS(ESI): *m/z* = 383 $[M-H]^-$

Data for **13a**:[10] ^{1}HNMR (500MHz, $CDCl_3$): 3.96 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.65 (t, *J* = 4.3 Hz, 4H), 3.38 (s, 2H, CH_2), 2.43 (t, *J* = 4.3 Hz, 4H), 2.16 (s, 3H, CH_3). Spectroscopic data are according to the literature. [10]

Data for **13b**:[25] ^{1}HNMR (400MHz, $CDCl_3$): 4.01 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 3.32 (s, 2H, CH_2), 2.36 (brs, 4H), 2.12 (s, 3H, CH_3), 1.53 (brs, 4H), 1.37-1.42 (m, 2H). Spectroscopic data are according to the literature. [25]

4.2. DPPH free radical antioxidant activity

In vitro antioxidant activities were measured against 2,2-diphenyl-1-picrylhydrazyl (DPPH).[26] The values of IC_{50}, the effective concentration at which 50% of radicals were scavenged, were tested to evaluate the antioxidant activities. Generally, a lower IC_{50} values indicated higher antioxidant activity, the IC_{50} of commercial Coenzyme Q_0 and Coenzyme Q_{10} were measured for comparison. Each sample solution (4 mL) in anhydrous ethanol at different concentrations (0.05，0.1，0.2，0.4 and 0.8mg/ml) was added to the solution (4 mL, 0.05 mg/ml) of DPPH in anhydrous ethanol. The reaction mixture was incubated at 30 ℃. The scavenging activity on DPPH free radical was determined by measuring the absorbance at 517 nm after 30 min. The scavenging activity was expressed as a percentage of scavenging activity on DPPH:

scavenging activity (%) = $[(A_{control} - A_{test}) / A_{control})] \times 100$

where $A_{control}$ is the absorbance of the control (DPPH solution without test sample)

and A_{test} is the absorbance of the test sample (DPPH solution plus scavenger). The control contains all reagents except the scavenger. The DPPH radical scavenging activity of Coenzyme Q_0 and Coenzyme Q_{10} were also assayed for comparison, all tests were performed in triplicate. Percent inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC_{50} value, the data caculated for antioxidantion are presented as means $\pm$ SD of three experiments.

Acknowledgments

This study was supported by the National Natural Science Foundation for Young Scientists of China, the Natural Science Foundation of Jiangsu Province (BK20160443), the College students practice innovation training program of Yancheng Teachers University, the Natural Science Foundation of Yancheng Teachers University.

References

[1] a) Y. Zhang, Y. Fang, H. Liang, H. Wang, K. Hu, X. Liu, X. Yi, Y. Peng, Bioorg. Med. Chem. Lett. 23 (2013) 107-111; b) G. Lenaz, M.L. Genova, *Biochim. Biophys. Acta.* **2009**, *1787*, 563-573

[2] a) M. Bentinger, K. Brismar, G. Dallner, Mitochondrion. 7 (2007) S41-50; b) M. Bentinger, M. Tekle, G. Dallner, *Biochem. Biophys. Res. Commun.* **2010**, *396*, 74-79.

[3] J. Liu, L. N. Wang, *CNS. Drugs.* **2014**, *28*, 63-68.

[4] M. Mancuso, D. Orsucci, L.Volpi, V. Calsolaro, G. Siciliano, *Current. Drug.*

Targets. **2010**, *11*,111-121.

[5] D.S. Kerr, *Neurotherapeutics*. **2013**, *10*, 307-319.

[6] EM. Yubero-Serrano, L. Gonzalez-Guardia, O. Rangel-Zuñiga, J.Lopez-Miranda, *Age (Dordr)*. **2013**, 35, 159-170.

[7] Z. Pei, T. Gustavsson, R. Roth, T. Frejd, C. Hägerhäll, *Bioorg. Med. Chem.* **2010**, 18, 3457-3466.

[8] A.M. James, H.M. Cochemé, M. Murai, H. Miyoshi, M.P. Murphy. *FEBS J.* **2010**, 277, 2067-2082

[9] Y.S. Jung, B.Y. Joe, S.J. Cho, Y. Konishi, *Bioorg. Med. Chem. Lett.* **2005**, 15, 1125-1129.

[10] K. Okamoto, M. Matsumoto, M. Watanabe, M. Kawada, T. Imamoto, I. Imada, *Chem. Pharm. Bull.* **1985**, 33, 3745-3755.

[11] H. Yabunaka, A. Kenmochi, Y. Nakatogawa, K. Sakamoto, H. Miyoshi, *Biochim. Biophys. Acta.* **2002**, 1556, 106-112.

[12] W. Ma, H. Zhou, Y.L. Ying, D.W. Li, G.R. Chen, Y.T. Long, H.Y. Chen, *Tetrahedron.* **2011**, 67, 5990-6000.

[13] Y. Toyokichi, E. Masakazu, T. Masato, S. Shohei, E. Kenichi, F. Hiroko, Y. Mie, Y. Kumio, **1989**, JP01319453

[14] M.P. Crozet, L. Giraud, J.R. Sabuco, P. Vanelle, M. Barreau, *Tetrahedron. lett.* **1991**, 32, 4125-4128.

[15] A. Giraud, P. Vanelle, L. Giraud, *Tetrahedron. lett.* **1999**, 4321- 4322.

[16] W. Oettmeier, R. Dostatni, H.J. Santel, Z. Naturforsch. **1987**, 42c, 693-697

[17] a) J. Wang, J.Yang, B.Yang, J.Q. Sun, T. Yang, *J. Chem. Res.* **2010**, *34*,724-725; b) J. Wang, J.Yang, B.Yang, X. Hu, J.Q. Sun, T. Yang, *J. Chem. Res.* **2010**, *34*, 717-718; c) J. Wang, J.Yang, R. G. Zhou, B. Yang, Y. S. Wu, *J. Chem. Res.* **2011**, *35*, 431-432; d) J. Wang, J.Yang, R. G. Zhou, B. Yang, Y. S. Wu, *J. Chem. Res.* **2011**, *35*, 428-430; e) J. Wang, R.G. Zhou, T. Liao, J. Yang. *Res. Chem. Intermed.*, **2015**, 41, 6393-6396. f) J. Wang, S. Li, T. Yang, J. Yang. *Eur. J. Med. Chem.*, **2014**, 86, 710-713. g) J. Wang, X. Hu, J. Yang, *Synthesis*, **2014**, 46, 2371-2375; h) J. Wang, S. Li, X. Hu, J. Yang, *Org. Prep. Proced. Int.*, **2014**, 46, 469-474. i) J. Wang, S. Li, T. Yang, J. Yang. *Tetrahedron.* **2014**, 70. 9029-9032.

[18] a). A. Bali, S. Malhotra, H. Dhir, A. Kumar, and A. Sharma, *Bioorg. Med. Chem. Lett.* **2009**, 19, 3041-3044; b) D. E. Bergbreiter, P. L. Osburn, and C. Li, *Org. Lett.* **2002**, 4, 737-740

[19] B.L. Wang, X.H. Liu, X.L. Zhang, J.F. Zhang, H.B. Song, Z.M. Li, *Chem. Biol. Drug. Des.* **2011**, 78, 42-49

[20] B. H. Lipshutz, A. Lower, V. Berl, K. Schein, and F.Wetterich, *Org. Lett*. **2005**, **7**, 4095-4097.

[21] M. Lim, C. Murray, T. Tronic, K. deKrafft, A. Ley, J. deButts, R. Pike, H. Lu, and H. Patterson, *Inorg. Chem.* **2008**, 47, 6931-6947.

[22] T. Wang, Z. Zhang, N. A. Meanwell, *J. Org. Chem.* **1999**, *64*, 7661-7662

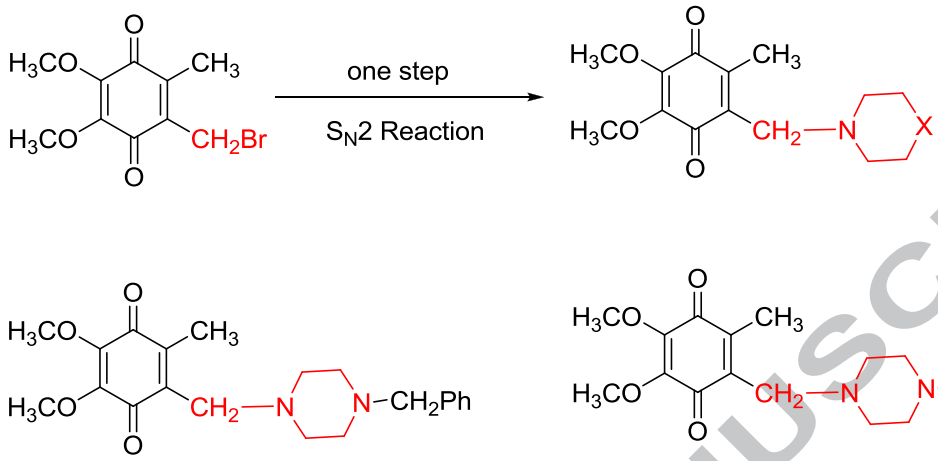
[23] W. Ma, D.-W. Li, T.-C. Sutherland, Y. Li, Y.-T. Long, H.-Y. Chen, *J. Am. Chem. Soc.* 2011, 133, 12366-12369.

[24] H. Matsumoto, Y. Niiro, T. Satoh, H. Ueda, **1996**, WO1996005202 A1

[25] J. Yang, Study on synthesis of Coenzyme Q analogues, thesis, Si Chuan University, **2006**, China.

[26] Ma, L.; Xiao, Y.; Li, C.; Xie, Z.-L.; Li, D.-D.; Wang, Y.-T.; Ma, H.-T.; Zhu, H.-L.; Wang, M.-H.; Ye, Y.-H., *Bioorg. Med. Chem.* **2013,** *21* (21), 6763-6770

Graphical abstract



I IC_{50} = 0.609 mM

13c IC_{50} = 0.294 mM

Efficient Synthesis and antioxidant activities of N-heterocyclyl substituted Coenzyme Q analogues

Highlights

1. A new strategy for the efficient synthesis of C-5 heterocyclyl substituted Coenzyme Q analogues was developed.
2. All the N-heterocyclyl substituted Coenzyme Q analogues are exhibiting good antioxidant properties.