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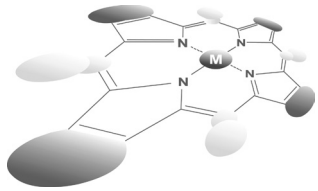

A facile synthesis and application of protoporphyrin derivatives on reducing the tobacco specific *N*-nitroamines levels of smoke

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ABSTRACT: Two protoporphyrin derivatives were prepared by a facile method using inexpensive hemin as starting material. They were added to cigarette filters to reduce the carcinogenic tobacco specific *N*-nitroamines (TSNAs), especially toward NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and NNN (*N*-nitrosonornicotine) for environment protection and public health. The reduction level of TSNAs was reached to 37.6% from MSS, with greater reductions when more porphyrin was included in the filter. The decrease level for NNK by protoporphyrin derivatives is more effective than NNN. The interaction between protoporphyrin derivatives and TSNAs (NNK and NNN) were investigated by fluorescence spectra and UV-visible titration. The correlation coefficients were 0.978~0.997 and the binding constants was the scope from 1.26×10^3 to 4.04×10^4. The interaction mechanisms between protoporphyrin derivatives and, NNK and NNN are possibly the co-interaction of hydrogen bond binding and strong π–π stacking.

KEYWORDS: protoporphyrin derivatives, hemin, tobacco-specific *N*-nitrosamines, binding constant, cigarette smoke.

INTRODUCTION

There are more than 1.2 billion smokers worldwide and more than 4 million of them die annually from smoking-related disease [1]. Multiple epidemiological studies have shown that cigarette smoke is related to the development of cardiovascular disease, stroke, lung carcinoma, chronic bronchitis, chronic obstructive pulmonary disease, and emphysema [2]. Cigarette smoking is causally related to the cancers of the lung, oral cavity, larynx, pharynx, nasal cavity, esophagus, liver, pancreas, kidney, urinary bladder, cervix, and myeloid leukemia [3–5]. Cigarette smoke is a complex mixture of more than 4700 chemicals and contains hundreds of toxicants.

Most of the nitroamines are characterized with functional group of N–NO, and tobacco-specific nitrosamines (TSNAs) are the carcinogenic agents identified in tobacco and tobacco smoke [6, 7]. *N*-nitrosoanabasine (NAB), *N*-nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and *N*-nitrosonornicotine (NNN) are the main components of TSNAs (Fig. 1). They are formed during the curing, processing, fermentation, and combustion of tobacco [8–10].

TSNAs play an important role as causative agents in cancer of the esophagus, pancreas, and oral cavity associated with smoking. Among the TSNAs, NNK and NNN are the most carcinogenic ones in laboratory animal [11, 12]. NNK induces lung tumors in rodents

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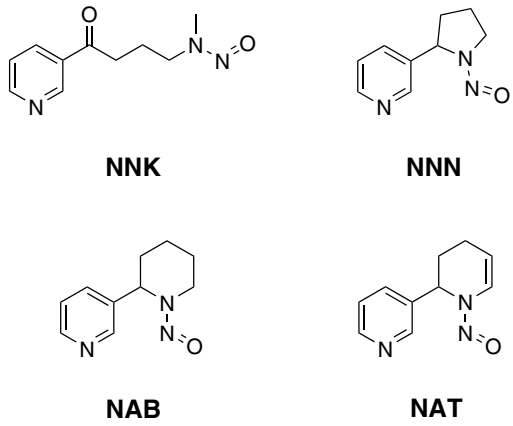


Fig. 1. Chemical Structure of typical TSNAs

independent of the route of administration [13]. NNN induces tumors of the esophagus and nasal cavity in rats, the lung in mice, the respiratory tract in hamsters, and the nasal cavity in mink [13, 14]. A mixture of NNK and NNN produced oral cavity tumors in rats [15]. According to the International Agency for Research on Cancer, NNK and NNN are carcinogenic to humans [16]. Consequently, in order to protect environment and public health, it is necessary to reduce the level of nitrosamines in environmental tobacco smoke and decrease the content of TSNAs, specially, reducing the level of NNK and NNN.

The removal of tobacco-specific nitrosamines (TSNAs) from cigarette smoke has attracted growing interest over the past years in tobacco chemistry [17–21]. Different methods were adopted to reduce TSNAs. Different additives were put into cigarette filter to reduce the nitrosamine level of the smoke, which is applied widely in cigarette industry. Previous studies have indicated the removal of carcinogenic TSNAs from cigarettes smoke by adding zeolite-like calcosilicate [22, 23]. Although zeolite-like calcosilicate effectively reduced TSNAs level in mainstream smoke (MSS), it affected the inhaled taste feeling and it is also expensive for large scale application. Some γ-AlOOH superstructures were prepared and added to the filter and/or cut fillers. The decrease in the amounts of four TSNAs in cigarette smoke by adding three-quarter-sphere-like γ-AlOOH superstructures into cut tobacco was very effective, but its harsh conditions (high temperature and high pressure) for preparation limited its further application in large scale [24].

Hemoglobin and some porphyrins have been shown to be promising cigarette filter additives to remove the hazardous components, such as carcinogenic *N*-nitroso compounds [25]. The compounds containing porphyrin structures play important roles in photosynthesis, gas transport (hemoglobin, myoglobin), vitamin structure (cobalamin), and the metabolism of living organisms. Examples include heme, which is the iron porphyrin in hemoglobin, and chlorophyll, which is a magnesium porphyrin [26]. Owing to their unique structures, porphyrins have a wide range of applications in bionics, materials chemistry, pharmaceutical chemistry, electrochemistry, optical physics and chemistry, as well as analytical chemistry [27–31]. In our previous study [32], tetraphenylporphyrin compounds were applied in tobacco industry to effectively decrease the harmful components such as TSNAs and B[a]P in MSS. Its low yields for the large scale preparation limited its further application. This encourages us to further investigate alternative porphyrin compounds for the application to reduce the harmful components in cigarette smoke.

In this study, the protoporphyrin derivatives were prepared using inexpensive hemin as starting materials by a facile synthesis, and the derivatives were added to cigarette filters to investigate the variation of the NNK and NNN level in the main stream smoke (MSS). The hemin had been applied widely in pharmaceutical industry, food industry with aboundant source and low cost. The interactions of protoporphyrin derivatives with NNK and NNN were investigated using fluorescence spectra and UV-vis titration. A possible mechanism for the reduction of TSNAs level by protoporphyrin derivatives is discussed.

EXPERIMENTAL

General

NMR spectra were measured on a Bruker AMX-400. The ^{1}H NMR (400 MHz) chemical shifts were given in ppm relative to internals reference TMS. ESI-MS and HR-MS spectral data were recorded on a Finnigan LCQ^{DECA} and a Bruker Daltonics BioTOF mass spectrometer, respectively. Fluorescence excitation and emission spectra were obtained using Fluoro Max-4 Spectrofluorophotometer (HORIBA Jobin Yvon). UV-vis absorption spectra were recorded on a Hitachi PharmaSpec UV-1900 UV-visible spectrophotometer. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification.

Standardized NNK, NNN were obtained from Sigma-Aldrich (St. Louis, MO). Hemin was extracted from animal fresh blood in our laboratory [33]. All of the solvents were dried according to the standard methods prior to use. All of the solvents were either HPLC or spectroscopic grade in the optical spectroscopic studies. TLC analyses were performed on silica gel GF254.

Synthesis of protoporphyrin derivatives

Synthesis of CY-1. To the round-bottom flask hemin (10.0 g, 15.34 mmol) and 100 mL of 33% HBr in HOAc was added, and the mixture was stirred at room temperature for 48 h. The resulting bromo-substituted protoporphyrin solution was used directly in the next step. To the above

solution, methanol (500 mL) was added slowly and the solution was refluxed for 8 h. Then the solvent was removed *in vacuo*, and the residue was suspended in water and the pH was adjusted to 8–9 with 2 M NaOH aq. solution. The solid was collected by filtration to give crude **CY-1**. The crude product was recrystalized from the mixture solvent of ethyl acetate and petroleum ether to afford **CY-1**[34] (7.6 g, 11.61 mmol) with 75.7% yield. ^{1}H NMR ($CDCl_3$): δ, ppm -3.66 (s, 2H, pyrrole-H), 2.28 (d, 6H, *J* = 4.4 Hz, CH$\underline{CH_3}$), 3.31 (m, 4H, $\underline{CH_2}$COO), 3.64–3.37 (m, 24H, OCH_3, CH_3), 4.43 (m, 4H, $\underline{CH_2}CH_2COO$), 6.07 (m, 2H, $CH_3\underline{CH}$), 10.10, 10.14, 10.53, 10.57 (4s, 4H, *meso*-H). ESI MS (positive ion mode) (rel. int.): *m/z* 655 ([M + H]$^+$, 100). HRMS (ESI): *m/z* 655.3469 ([M + H]$^+$, 100); calcd. for $C_{38}H_{46}N_4O_6$ + H 655.3417 ([M + H]$^+$, 100). UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 399 (5.27), 498 (4.15), 532 (3.95), 569 (3.77) and 621 (4.62). IR: ν, cm^{-1} 3313.0, 2971.0, 2925.0, 2860.4, 1741.7, 1455.6, 1362.5, 1168.1, 1112.5, 1085.1, 964.9, 826.1, 743.0 and 678.5.

Synthesis of CY-2. To the round-bottom flask **CY-1** (4.0 g, 6.11 mmol) and 200 mL of 1 M NaOH methanol solution was added, and the mixture was refluxed for 2 h. TLC monitored the disappearance of **CY-1**. Then the solvent was removed *in vacuo*, and the residue was dissolved in water then the pH was adjusted to 4–5 with AcOH to give **CY-2** [35] (3.56 g, 5.68 mmol) with 93.0% yield. ^{1}H NMR (DMSO-d_6): δ, ppm -3.97 (s, 2H, pyrrole-H), 2.18 (d, 6H, *J* = 5.6 Hz,CH$\underline{CH_3}$), 3.07 (t, 4H, *J* = 8.4 Hz, $\underline{CH_2}$COO), 3.60–3.69 (m, 18H, OCH_3, CH_3), 4.32 (t, 4H, *J* = 8.4 Hz, $\underline{CH_2}CH_2COO$), 6.15 (q, 2H, *J* = 5.6 Hz, $CH_3\underline{CH}$), 10.19, 10.43, 10.51, 10.58 (4s, 4H, *meso*-H). ESI MS (positive ion mode) (rel. int.): *m/z* 627 ([M + H]$^+$, 100). HRMS (ESI): *m/z* 627.3170 ([M + H]$^+$, 100), calcd. for $C_{36}H_{42}N_4O_6$ + H 627.3104 ([M + H]$^+$, 100). UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 397 (5.02), 498 (3.99), 532 (3.81), 568 (3.68) and 619(3.40). IR: ν, cm^{-1} 3424.3, 3304.1, 2989.5, 2906.4, 2869.3, 2804.8, 1704.5, 1556.2, 1399.6, 1260.9, 1177.8, 1103.6, 1066.5, 973.8, 826.1, 743.0 and 668.9.

Synthesis of CY-3 [36]. The preparation method of **CY-3** is the same with that of **CY-1** except using n-butanol as substrate, and the yield was 71.3%. ^{1}H NMR ($CDCl_3$): δ, ppm -3.70 (s, 2H, pyrrole-H), 0.71–0.75 (t, 6H, *J* = 7.2 Hz, $OCH_2CH_2CH_2\underline{CH_3}$), 0.78–0.86 (m, 6H, $OCH_2CH_2CH_2\underline{CH_3}$), 1.19 (q, 4H, *J* = 7.2 Hz, $OCH_2CH_2\underline{CH_2}CH_3$), 1.42–1.50 (m, 8H, $OCH_2\underline{CH_2}\underline{CH_2}CH_3$), 1.73–1.77 (m, 4H, $OCH_2\underline{CH_2}$-CH_2CH_3), 2.25 (d, 6H, *J* = 6.8 Hz, CH$\underline{CH_3}$), 3.28 (q, 4H, *J* = 6.4 Hz, $\underline{CH_2}$COO), 3.64–3.75 (m, 16H, CH_3, OCH_2-), 4.08 (q, 4H, *J* = 6.4 Hz, $O\underline{CH_2}CH_2CH_2CH_3$), 4.42 (t, 4H, *J* = 6.4 Hz, $\underline{CH_2}CH_2COO$), 6.10 (q, 2H, *J* = 6.8 Hz, $CH_3\underline{CH}$), 10.09, 10.11, 10.61, 10.62 (4s, 4H, *meso*-H). ESI MS (positive ion mode) (rel. int.): *m/z* 823 ([M + H]$^+$, 100). HRMS (ESI): *m/z* 823.5387 ([M + H]$^+$, 100); calcd. for $C_{50}H_{70}N_4O_6$ + H 823.5295 ([M + H]$^+$, 100). UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 400 (5.37), 499 (4.33), 533 (4.14), 568 (4.01) and 621(3.81). IR: ν, cm^{-1} 3435.2, 3310.7, 2959.0, 2929.5, 2867.7, 1733.6, 1610.2, 1455.6, 1387.2, 1230.3, 1166.2, 1097.6, 967.6, 834.8 and 740.0.

Synthesis of CY-4. To the round-bottom flask **CY-3** (4.0 g, 5.86 mmol) and 100 mL of 1 N NaOH methanol solution was added, and the mixture solution was refluxed for 4 h. TLC monitored the disappearance of **CY-3**. Then the solvent was removed *in vacuo* and the residue was dissolved in water then the pH was adjusted to 4–5 with AcOH to give **CY-4** (3.10 g, 4.36 mmol) with 89.7% yield. ^{1}H NMR (DMSO-d_6): δ, ppm -3.93 (s, 2H, pyrrole-H), 0.78 (t, 6H, *J* = 7.2 Hz, $OCH_2CH_2CH_2\underline{CH_3}$), 1.38–1.46 (m, 4H, $OCH_2CH_2\underline{CH_2}CH_3$), 1.66–1.72 (m, 4H, $OCH_2\underline{CH_2}CH_2CH_3$), 2.16 (d, 6H, *J* = 6.4 Hz, CH$\underline{CH_3}$), 2.94 (t, 4H, *J* = 7.2 Hz, $\underline{CH_2}$COO), 3.66–3.75 (m, 16H, CH_3,OCH_2-), 4.34 (t, 4H, *J* = 7.2 Hz, $\underline{CH_2}CH_2COO$), 6.15 (q, 2H, *J* = 6.4 Hz,$CH_3\underline{CH}$), 10.22, 10.60, 10.65, 10.80 (4s, 4H, *meso*-H). ESI MS (positive ion mode) (rel. int.): *m/z* 711 ([M + H]$^+$, 100). HRMS (ESI): *m/z* 711.4062 ([M + H]$^+$, 100); calcd. for $C_{42}H_{54}N_4O_6$ + H 711.4043 ([M + H]$^+$, 100). UV-vis (CH_2Cl_2): λ_{max}, nm (log ε) 399 (5.12), 497 (4.13), 529 (3.99), 568 (3.95) and 618 (3.67). IR: ν, cm^{-1} 3442.8, 3304.1, 2952.4, 2915.3, 2869.3, 1713.4, 1445.6, 1371.4, 1223.8, 1159.2, 1094.0, 844.7, 733.4 and 687.4.

Addition and analysis of protoporphyrin derivatives CY-2 and CY-4 to cigarettes filters

Cigarette filters consisting of acetate cellulose and cut filters of brand A were obtained from Chengdu Cigarette Factory (Chengdu, China). During machine molding of the filters, protoporphyrin derivatives were added to the filter through the carrier. Protoporphyrin derivatives-modified filters were then attached to cigarettes. Modified cigarettes were produced containing 0.0, 5.0, 15.0, 30.0, and 45.0 μg of the protoporphyrin derivatives per filter. Control cigarettes were prepared using unmodified acetate cellulose filters of brand A. Cigarettes containing protoporphyrins derivatives were used to investigate the reduction of TSNAs (NNK and NNN) in MSS (main stream smoke).

A fully automatic smoking machine (RM200, Borgwaldt Technik GmbH, Hamburg, Germany) was used to generate and trap MSS from the 20 cigarettes. NNK and NNN were detected by gas chromatography-thermal energy analysis spectrometry with an Agilent 6890 GC and a Thermo 610 TEA (Thermo Fisher Scientific, Waltham, MA) [37]. Gas chromatography mass spectrometry and gas chromatography thermal energy analysis spectrometry results were used to calculate the percentage reductions in TSNAs in the MSS from protoporphyrin derivatives modified cigarettes in comparison with the control cigarettes.

Mechanism studies

The interaction between protoporphyrin derivatives and TSNAs (NNK and NNN) were studied by fluorescence

spectra using Fluoro Max-4 Spectrofluorophotometer (HORIBA Jobin Yvon). A stock solution of the guest NNK and NNN, and host protoporphyrin derivatives **CY-2** and **CY-4** were prepared in dichloromethane. The concentrations of NNK and NNN solutions were 1.45 mM and 3.61 mM, respectively. The concentrations of protoporphyrin derivatives (**CY-2** and **CY-4**) were 5.74 μM and 4.92 μM respectively. The fluorescence intensity was measured by the addition of different volumes TSNAs to 3 mL of host protoporphyrin derivative solution. The fluorescence intensity for different concentrations of TSNAs were plotted to obtain the protoporphyrin derivatives fluorescence spectrum.

The fluorescence change of protoporphyrin derivatives after the addition of TSNAs conforms to the Benesi-hildebrend equation (Equation 1) [38].

$$\frac{1}{A - A_0} = \frac{1}{K(A_{\max} - A_0)[TSNAs]} + \frac{1}{A_{\max} - A_0} \quad (1)$$

in which A_0 is the absorance of protophyrin derivatives, A is the absorbance obtained with TSNAs, A_{max} is the absorbance obtained with excess amount of TSNAs, K is the association constant, and [TSNAs] is the concentration of TSNAs added. The binding constant K and the interaction between the protoporphyrin derivatives and TSNAs are positively correlated. Larger binding constants indicate stronger interaction between the protoporphyrin derivatives and TSNAs.

The interaction between protoporphyrin derivatives and, NNK and NNN was also investigated by UV-visible spectrophotometer (Hitachi PharmaSpec UV-1900). Various concentrations of NNK and NNN (2×10^{-3} M) were added to the solution of host protoporphyrin derivatives (4×10^{-6} M) and the mixture was vortexed for 10 s, then the spectrum were recorded.

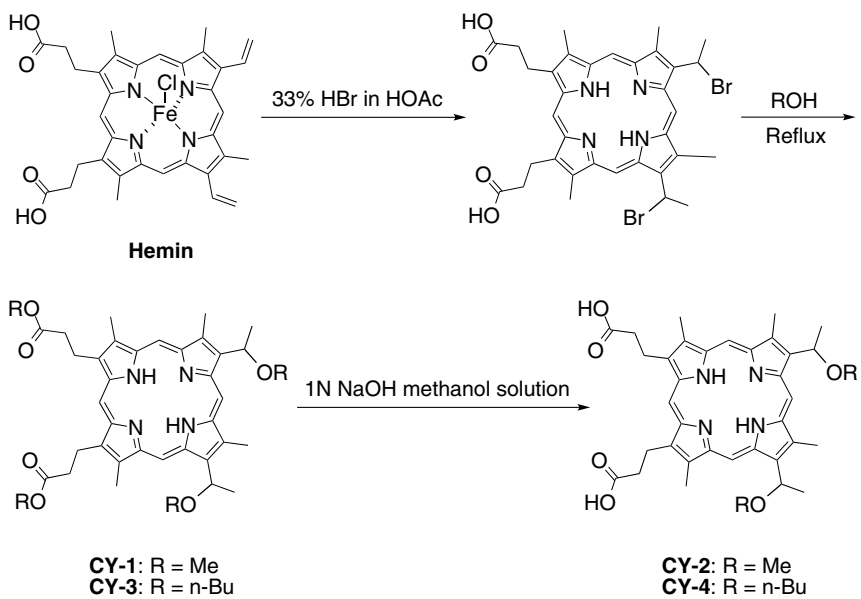
RESULTS AND DISCUSSION

Synthesis of protoporphyrins derivatives

In this study, protoporphyrin derivatives **CY-2** and **CY-4** were prepared using cheap and abundant hemin as starting material. The deferrous reaction of porphyrin center and HBr addition on double bond were carried out in a "one pot reaction" (Scheme 1). Especially, the bromo-substituted compounds need not be purified and can be directly used. In the following step using different alcohols as reactant, the nucleophilic substitution of Br group by alkyloxy group is associated with the esterification of carboxylic acid. The titled compounds could be easily obtained by simple saponification by NaOH. Only easy operations such as extraction, acidification, filteration and recrystallization were needed to obtain the pure products. In the preparation of **CY-2**, less amount of demonomethyl by-product was detected, and it was easy to be removed by recrystallization. In other words, the whole process was convenient and efficient. It is promising for large scale application in the future.

Reduction of TSNAs in MSS by the addition of protoporphyrin derivatives CY-2 and CY-4

NNK and NNN in MSS were greatly reduced by adding **CY-2** and **CY-4** compounds to the cigarette filters (Table 1). The addition of protoporphyrin derivatives **CY-2** and **CY-4** reduced the levels of TSNAs in MSS by up to 36.4% and 37.6%. Generally, more



Scheme 1. Synthetic route for protoporphyrin derivatives **CY-2** and **CY-4**

Table 1. Percentage reduction (%) in the levels of NNK & NNN in MSS (n = 5) after the addition of protoporphyrin derivatives **CY-2** & **CY-4** to the cigarette filter[a]

CY-2 (μg)	NNK	NNN	CY-4, μg	NNK	NNN
0	0	0	0	0	0
5	13.6	10.7	5	20.4	10.5
15	22.9	13.9	15	23.6	14.8
30	33.8	21.9	30	33.2	25.1
45	36.4	22.5	45	37.6	26.8

[a] The percentage reduction was calculated in comparision to the level in control cigarettes with unmodified filters.

reduction of TSNA levels was achieved for cigarettes containing more amounts of protoporphyrin derivatives in the filter. With regard to both **CY-2** and **CY-4**, the reduction of NNK is more effective than the reduction of NNN.

Interaction between protoporphyrin derivatives and NNK and NNN

Fluorescence spectra (Fig. 2) of **CY-2** and **CY-4** were obtained with various doses of NNK and NNN. The fluorescence intensity of protoporphyrin derivatives **CY-2** and **CY-4** increased with the increase of the addition amount of NNK and NNN. According to Equation 1, the linear equation, coefficient, and slope (K) were obtained from the $1/A–A_0$ and 1/[TSNAs] concentration curve (Fig. 3). The plot of $1/(A–A_0)$ against 1/[TSNAs] show a linear relationship, indicating that protopotphyrin derivatives associates with TSNAs in 1:1 stoichiometry. The association constant K between protoporphyrin and TSNAs is determined from the slope and intercept of liner equation.

The binding constant (K) between **CY-2** and, NNK and NNN were 6.95×10^3 and 1.26×10^3 with the correlation coefficient (r) of 0.997 and 0.982, respectively. The binding constant (K) between **CY-4** and, NNK and NNN were 4.04×10^4 and 2.34×10^3 with the correlation coefficient (r) of 0.988 and 0.978, respectively, (Fig. 3). The binding constants indicate that the interaction between the protoporphyrin derivatives **CY-2** and **CY-4** and, NNK and NNN was strong. Relatively larger binding constants were observed between **CY-2** and **CY-4,** and NNK. Therefore, the interaction between the protoporphyrin derivatives and NNK is stronger than those involving NNN. These results were consistent with the reduce level of TSNAs in MSS.

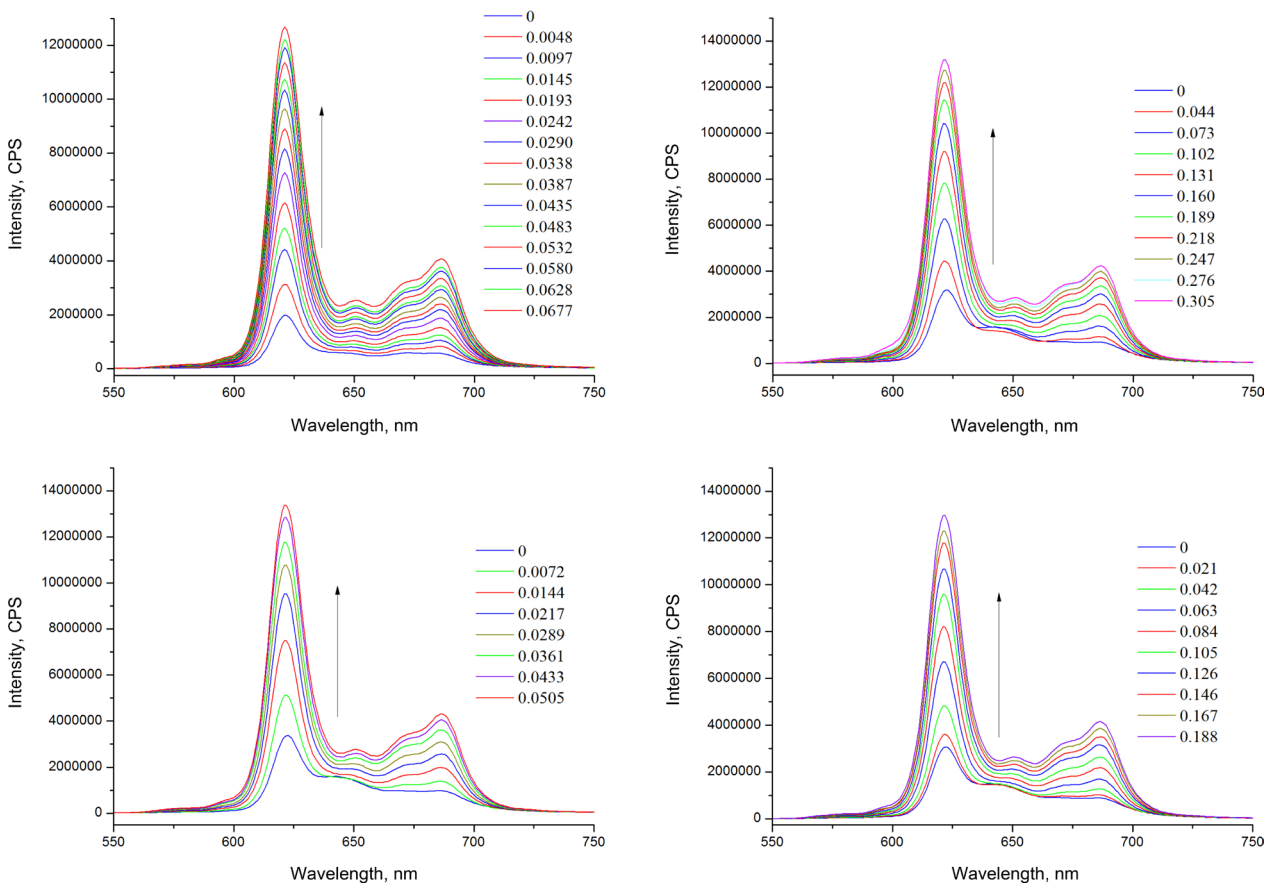


Fig. 2. Changes in fluorescence emission spectra of the protoporphyrin derivatives **CY-2** (upper) and **CY-4** (lower) in dichloromethane with various concentrations (mM) of NNK (left) & NNN (right)

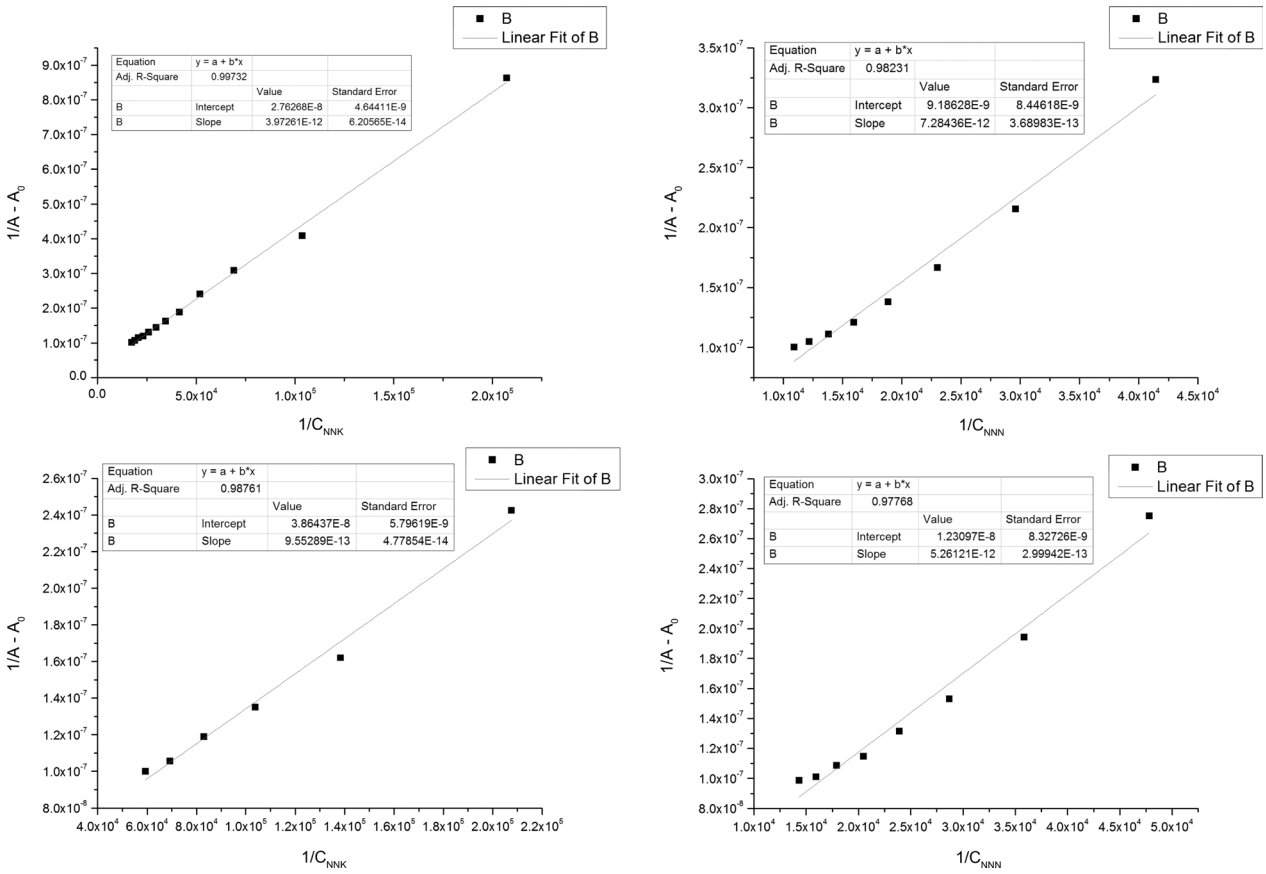


Fig. 3. B-H fluorescence plot of **CY-2** (upper) and **CY-4** (lower) with NNK (left) and NNN (right)

The UV-visible spectra (Fig. 4) of protoporphyrin derivatives **CY-2** and **CY-4** with different concentrations of NNK and NNN were obtained. The intensity of the characteristic absorption peak of protoporphyrin derivatives **CY-2** and **CY-4** at 399 nm enhanced along with the increase of the TSNA concentrations, finally reaching saturated status. The spectral intensities for the different concentrations of TSNAs were plotted to obtain the protoporphyrin derivatives UV-vis spectrum. A slight red shift (about 2 nm) was observed in the UV-vis spectra. UV-visible spectrum experiments suggest that protoporphyrin derivatives **CY-2** and **CY-4** could interact strongly with NNK and NNN.

Mechanism of TSNAs reduction by protoporphyrin derivatives

Porphyrin molecules with the macrocyclic "tetrapyrrole" have rigid structures and large aromatic framework, specific binding of OH, NH_2 functionalities or other tailed ligands according to the hard-soft acid-base paradigm, and the precise control on the topology and substitution of the receptor through well-established synthetic methodologies that allow the prophyrin macrocycle to be decorated on demand. Many porphyrin compounds were designed to specifically recognize particular molecules with certain molecular sizes, properties, functional groups, and chirality. Strong π–π interactions may occur between porphyrins and aromatic molecules [39], and this property has been applied on the liquid chromatography stationary phase [40]. Meanwhile, the hydrogen bond interaction between prophyrins and small guest molecules was generally used to construct supermolecular compounds, and it plays important role in molecular recognition [41–42]. We speculate that protoporphyrin derivatives **CY-2** and **CY-4** may interact with NNK and NNN by the hydrogen binds between NO functional groups and OH. The π–π interactions between the macrocyclic structure of porphyrin and the aromatic framework of pyridine maybe another driving force.

CONCLUSION

Protoporphyrin derivatives are potent additives in cigarettes to reduce TSNAs levels in MSS and the environment. The addition of protoporphyrin to cigarette filters is economical, environmental friendly, and feasible for cigarette production. The results of this study could facilitate the development of low-harm cigarettes and reduce the levels of TSNAs in the environment to decrease indoor air pollution. However, the potential transfer of porphyrins to cigarette smoke or their dissolution in the

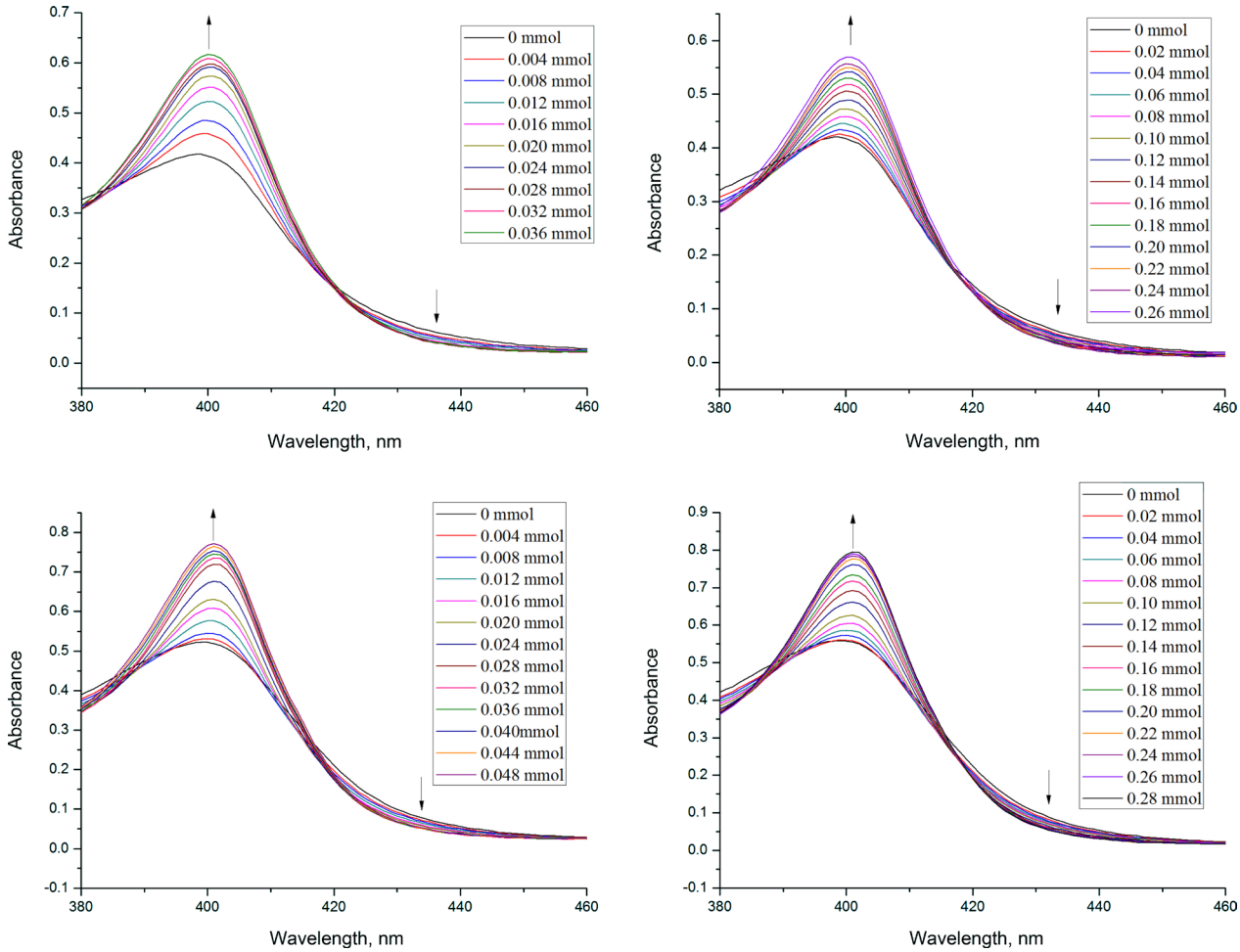


Fig. 4. Change in absorption spectra of **CY-2** (upper) and **CY-4** (lower) in DCM with various amount NNK (left) & NNN (right); the concentration of **CY-2** & **CY-4** was 4 μM

mouth and the toxicological consequences need to be further evaluated in future studies.

Acknowledgements

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Supporting information

<<Text to be completed by authors>> Supplementary material is available free of charge *via* the Internet at http://www.worldscinet.com/jpp/jpp.shtml.

REFERENCES

1. Corrao MA, Guindon GE, Sharma N and Shokoohi DF. *Tobacco Control Country Profiles*, American Cancer Society: Atlanta, Georgia, USA, 2000.
2. Tønnesen P and Vermeire PA. *Eur. Respir. J.* 2000; **16**: 1031–1034.
3. IARC monographs. *Tobacco Smoke and Involuntary Smoking* (IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans), International Agency for Research on Cancer (IARC): Lyon, France, 2002.
4. Doll R. *Br. Med. J*. 1996; **52**: 35–49.
5. Rodgman A and Perfetti TA. *The Chemical Components of Tobacco and Tobacco Smoke*, CRC Press: Boca Raton, Florida, USA, 2009.
6. Hoffmann D and Hecht SS. *Cancer Res*. 1985; **45**: 935–944.
7. Hecht SS and Hoffmann D. *Carcinogenesis* 1988; **9**: 875–884.
8. Hoffmann D, Brunnemann KD, Prokopczyk B and Djordjevic M. *J. Toxicol. Environ. Health* 1994; **41**: 1–52.
9. Spiegelhalder B and Bartch H. *Eur. J. Cancer Prev*. 1996; **5**: 33–38.
10. Adams JD, Lee SJ, Vinchkovski N, Castonguay A and Hoffmann D. *Cancer Lett*. 1983; **17**: 339–346.
11. Hecht SS and Hoffmann D. *Cancer Surv*. 1989; **8**: 273–294.

12. Stepanov I, Carmella SG, Hecht SS and Duca G. *J. Agric. Food Chem.* 2002; **50**: 2793–2797.
13. Hecht SS. *J. Natl. Cancer Inst.* 1999; **91**: 1194–1210.
14. Hecht SS. *Chem. Res. Toxicol.* 1998; **11**: 559–603.
15. Hecht SS, Rivenson A, Braley J, DiBello J, Adams JD and Hoffmann D. *Cancer Res*. 1986; **46**: 4162–4166.
16. IARC monographs. *Smokeless Tobacco and Some Tobacco-specific Nitrosamines* (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans), International Agency for Research on Cancer (IARC): Lyon, France, 2005.
17. Zeng FH, Li W, Zhou JH, Zhang YY and Hu ZM. *Chin. Agri. Sci. Bull.* 2010; **26**: 82–86.
18. Xie LY, Liu Q, Zhu XQ and Liu B. *Environ. Sci. & Technol.* 2006; **29**: 133–135.
19. Hou HW, Zhu Y, Tang GL, Hu QY, Yang L and Qin YH. *Tobacco Sci. & Technol.* 2011; **1**: 52–55.
20. Rundlf T, Olsson E, Wiernik A, Back S, Aune M, Johansson L and Wahlberg I. *J. Agric. Food Chem.* 2000; **48**: 4381–4388.
21. Li SP, Shu JS, Zhang CY, Lin H, Chen J and Yang J. *J. Anhui Agri. Sci.* 2010; **38**: 20472–20473.
22. Gao L, Cao Y, Zhou SL, Zhuang TT, Wang Y and Zhu JH. *J. Hazard. Mater.* 2009; **169**: 1034–1039.
23. Meier WM and Siegmann K. *Microporous Mesoporous Mater.* 1999; **33**: 307–310.
24. Zhu Y, Hou HW, Tang GL and Hu QY. *Eur. J. Inorg. Chem.* 2010; 872–878.
25. Deliconstantinos G, Villiotou V and Stavrides JC. *Anticancer Res*. 1994; **14**: 2717–2726.
26. Biesaga M, Pyrzynska K and Trojanowicz M. *Talanta* 2000; **51**: 209–224.
27. Mody TD. *J. Porphyrins* Phthalocyanines 2000; **4**: 362–367.
28. Ballut S, Naud-Martin D, Loock B and Maillard P. *J. Org. Chem.* 2011; **76**: 2010–2028.
29. Chen S, Fetzer JC and Meyerhoff ME. *Fresenius' J. Anal. Chem.* 2001; **3–4**: 385–392.
30. Armstrong NR. *J. Porphyrins Phthalocyanines* 2000; **4**: 414–417.
31. Noworyta KC, Kutner W, Wijesinghe CA, Srour SG and D'Souza F. *Anal. Chem.* 2012; **84**: 2154–2163.
32. Wang CG, Dai Y, Feng GL, He R, Yang WM, Li DL, Zhou XZ, Zhu LJ and Tan LL. *J. Agric. Food Chem.* 2011; **59**: 7172–7177.
33. Wang CG, Dai Y, Liu SM and Yang WM. *Food Science* 2010; **31**: 109–113.
34. Brunner H, Arndt MR and Treittinger B. *Inorg. Chim. Acta* 2004; **357**: 1649–1669.
35. Kim YS, Song R, Kim DH, Jun MJ and Sohn YS. *Bioorg. Med. Chem.* 2003; **11**: 1753–1760.
36. Xu SC, Hu BC, Cui QL, Sun CG and Liu ZL. *J. Chem. Res.* 2011; **35**: 420–423.
37. Xie FW, Jin YM, Zhao MY, Wang S, Liu KJ and Wu M. *In National Standards of the People's Republic of China GB/T 23228-2008*, General Administration of Quality Supervision, Inspection and Quarantine of the P.R.C. and Standardization Administration of the P.R.C.: Beijing, China, 2009; pp 1–9.
38. a) Benesi HA and Hildebrand JH. *J. Am. Chem. Soc.* 1949; **71**: 2703–2707. b) Shiraishi Y, Sumiya S, Kohno Y and Hirai T. *J. Org. Chem*. 2008; **73**: 8571–8574. c) Rodriguez-Caceres MI, Agbaria RA and Warner IM. *J. Fluoresc.* 2005; **15**: 185–190.
39. Hunter CA and Sanders JKM. *J. Am. Chem. Soc.* 1990; **112**: 5525–5534.
40. Xiao J, Kibbey CE, Coutant DE, Martin GB and Meyerhoff ME. *J. Liq. Chromatogr. Relat. Technol.* 2011; **19**: 2901–2932.
41. Deviprasad GR and D'Souza F. *Chem. Commun.* 2000; 1915–1916.
42. Kavakka JS, Heikkinen S and Helaja J. *Eur. J. Org. Chem.* 2008; 4932–4937.