Electronic supplementary material

A nano-detection-system based on chemical probe for early diagnosis of Atherosclerosis in situ

Qiaolei Wang[1], Rui Lou[1], Qianwen Yin[1], Ruhe Yang[1], Shengnan Li[1], Jie Zhou[1,*]

1 School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China

* E-mail addresses of corresponding authors: Jie Zhou: Jie_0822@163.com

1. EXPERIMENTAL SECTION

1.1 Hofmann degradation of 3,4,5-trihydroxybenzamide

In a 25 mL eggplant bottle, 300.9 mg 3,4,5-trihydroxybenzamide (M_1=169.14, n_1=1.78 mmol), was mixed with 2 mL ultra-pure water. Then, add 1 mL NaClO and 1.5 mL 20% NaOH to the mixture dropwise. The reaction system was refluxed at 85 ℃ for 5h. The progress of the reaction was constantly monitored by thin layer chromatography (TLC). After the reaction completed and was cooled to room temperature, transfer the reaction system to anhydrous methanol, followed by suction filtration. The filter cake was dried in vacuum drying overnight (50 ℃). After drying, the product ***1a*** was characterized by Fourier Transform Infrared Spectrometer (FTIR) and Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR).

1.2 Ammonialysis of 4-bromo-1,8-naphthalic anhydride (compound ***2a*** and ***2b***)

Accurately weighed 277.1 mg 4-bromo-1,8-naphthalic anhydride (M_2=277.07, n_2=1.00 mmol) and 186.2 mg compound ***1a*** (M_3=155.15, n_3=1.20 mmol) in a 25 mL round bottom flask, and added 5 mL absolute ethanol to uniformly disperse the mixture. In addition, accurately weighed 277.3 mg 4-bromo-1,8-naphthalic anhydride (n_4=1.00 mmol) and 142.6 mg guanidine hydrochloride (M_4=95.05, n_5=1.50 mmol) in another 25 mL round bottom flask, and added 5 mL absolute ethanol to uniformly disperse the mixture. The both systems reflux at 70 ℃ for 8h. Use TLC to monitor the progress of the processes. When the reactions were completed, the reaction systems were transferred to a large amount of ice water, respectively. After suction filtration, the resulting filter cakes were dried in vacuum (60 ℃) drying overnight. The obtained solids were separately compound ***2a*** and ***2b***, and the products were characterized by FITR and ^{1}H NMR.

1.3 Substitution of 4-bromine (compound ***3a*** and ***3b***)

In a 25 mL round bottom flask, 150.7 mg compound ***2a*** (M_5=414.21, n_6=0.36 mmol) and 186.2 mg guanidine hydrochloride (n_7=0.54 mmol), and 3 mL Methoxyethanol was added. The reaction was refluxed for 6h at 135 ℃. In another 25 mL round bottom flask, replace ***2a*** with 127.7 mg compound ***2b*** (M_6=354.59, n_8=0.36

mmol), and other treatments were as same as above. After the two reactions are completed, the reaction system is respectively transferred to a large amount of anhydrous DCM and suction filtered. The filter cake was dried under vacuum at 50 ℃ overnight. The obtained solids were separately compound ***3a*** and ***3b*** (TBNG and GNTB), and the products were characterized by FITR and ^{1}H NMR.

1.4 Determination of fluorescence spectrum

Scan the solutions of TBNG and GNTB at 300 μg/mL in the full band (200-800 nm) of the ultraviolet-visible light region (UV-Vis), respectively, and measure their maximum absorption wavelengths λ_{max1} and λ_{max2}. Fluorescence spectrophotometer was used to measure the fluorescence spectra of TBNG and GNTB. Set λ_{max1} and λ_{max2} as the excitation wavelengths λex_1 and λex_2 of the target compounds TBNG and GNTB, respectively. Repeat the measurement of excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) until they are unchanged. The obtained λ_{ex} and λ_{em} is the fluorescence wavelength of the target compound, and the corresponding spectral curve is the fluorescence spectrum of the target compound.

1.5 Determination of hydroxyl radical scavenging rate

In this experiment, the salicylic acid method was used to determine the hydroxyl radical scavenging rates of TBNG and GNTB. Accurately remove 60 μL different concentrations of the sample solutions (0, 10, 20, 30, 40, 60, 80, 100, 120 and 140 μg/mL), and then add 40 μL PBS (10 mmol/L), 30 μL $FeSO_4$ (0.4 mmoL/L), and 30 μL H_2O_2 (0.03 mmoL/L), shaking for 10min; add 30 μL 1,10-Phenanthroline monohydrate (0.35mmoL/L), shaking at 37 ℃ for 60min. The absorbance was measured at 510 nm and repeated three times, and the average was taken. In addition, Vc was used as a positive control and ultrapure water was used as a blank control.

The hydroxyl radical scavenging rate of the antioxidant is calculated according to the following formula:

$$Hydroxyl\ radical\ scavenging\ rate\ (\%) = \frac{A_0 - A_1}{A_0} \times 100\%$$

A_0 is the absorbance of the blank control; A_1 is the absorbance of the sample.

2. Results and Discussion

2.1 Characterization of TBNG and GNTB

The chemical formula of TBNG and GNTB are shown in Fig. S1. And the Infrared spectrum of TBNG, GNTB and the ingredients are shown in Fig. 2A and 2B.

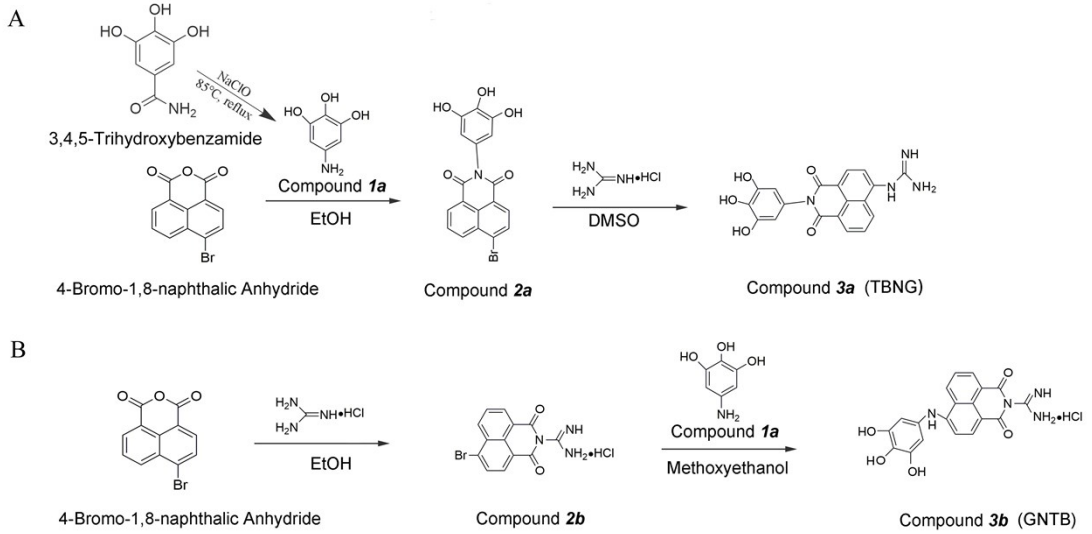

Fig. S1. The synthetic routes of chemical formula of TBNG and GNTB

(1) Characterization of TBNG (Fig. S2A): It can be seen from Fig. 1B that ***1a*** (the magenta line) has obvious characteristic peaks at 1591 cm^{-1} and 1316 cm^{-1}, and double peaks at 3351 cm^{-1} and 3347 cm^{-1}. These indicate that ***1a*** is a primary amine compound, that is, ***1a*** has been initially synthesized. ***2a*** (the dark cyan line) has a distinct broad peak at 3420 cm^{-1}, which corresponds to the O-H stretching vibration in phenolic compounds that form intermolecular hydrogen bonds, suggesting that ***2a*** contains the phenolic hydroxyl groups that can form intramolecular hydrogen bonds, while ***1a*** contains *o*-benzene triphenol structure, which means that ***2a*** contains a pyrogallol structure. The absorptions at 1223 cm^{-1} and 779 cm^{-1} correspond to the C-O stretching vibration and O-H out-of-plane bending vibration respectively, which also suggests that ***2b*** (Fig. S2B, the red line) contains phenolic hydroxyl groups. Furthermore, the absorption of ***2b*** at 1433 cm^{-1} corresponds to the C-N stretching vibration of the inner imide. In summary, it can preliminarily indicate that compound ***2b*** was successfully synthesized. In addition, ***3a*** (Fig. S2A, the red line) has a broad absorption peak at 3358 cm^{-1}, which corresponds to the O-H stretching vibration in phenolic compounds that form intermolecular hydrogen bonds, suggesting that the

structure of ***3a*** contains phenolic hydroxyl groups. Then, ***1b*** contains a pyrogallol structure, which means that ***3a*** contains a pyrogallol structure. Finally, compared with ***2a*** and 4-bromo-1,8-naphthalic anhydride (the blue line), ***3a*** has no absorption peak at 556 cm^{-1}, indicating that the Br atom in ***2a*** has been replaced. As a consequence, it can be preliminarily judged that the TBNG has been successfully synthesized. And ^{1}HNMR spectrum of TBNG are shown in Fig. S3.

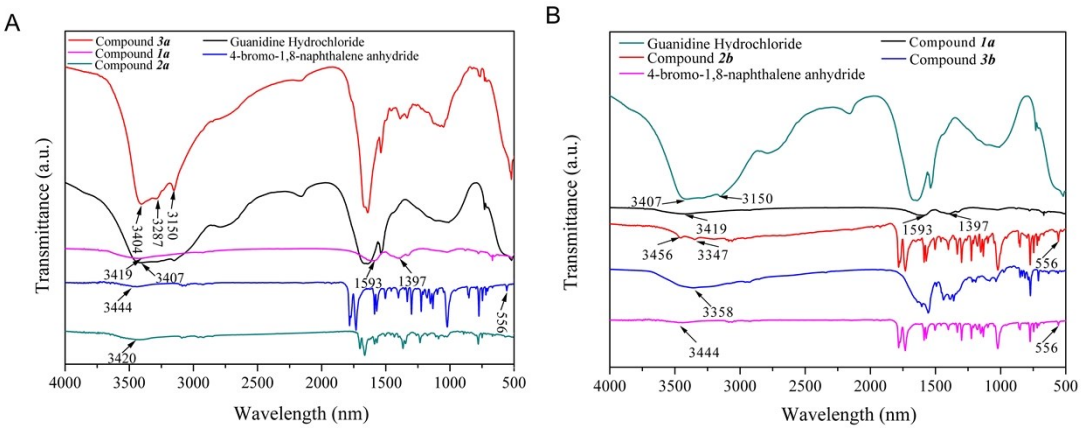

Figure S2 (A) Infrared spectrum of TBNG and the ingredients; (B) Infrared spectrum of GNTB and the ingredients.

(2) Characterization of GNTB (Fig. S2B): As shown in Fig. S2B, ***2b*** (the red line) has obvious absorption peaks at 1782 cm^{-1}, 1401 cm^{-1}, and 719 cm^{-1}, suggesting that it contains an imide structure [-C(O)-NR, R≠H]. The two absorption peaks at 3456 cm^{-1} and 3347 cm^{-1} suggest that ***2b*** belongs to the primary amines. After the ammonolysis of guanidine and naphthyl anhydride, there is still a free amino, so it can be preliminarily judged that the synthesis of ***2b*** is successful. There are two obvious absorption peaks at 3380 cm^{-1} and 3161 cm^{-1}, which can be preliminarily judged to contain primary amino (-NH_2). Compared with ***2b*** and 4-bromo-1,8-naphthalic anhydride (the magenta line), ***3b*** (the blue line) has no absorption peaks at 556 cm^{-1} and 569 cm^{-1}, indicating that the structure does not contain Br atoms and it can be preliminarily judged that GNTB has been synthesized. And ^{1}H NMR spectrum of GNTB is shown in Fig. S3.

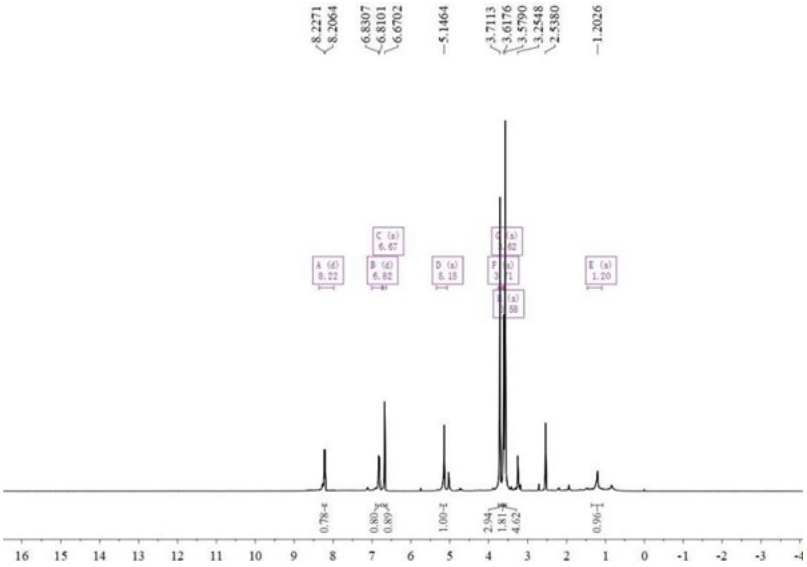

Fig. S3. ^{1}H NMR of TBNG

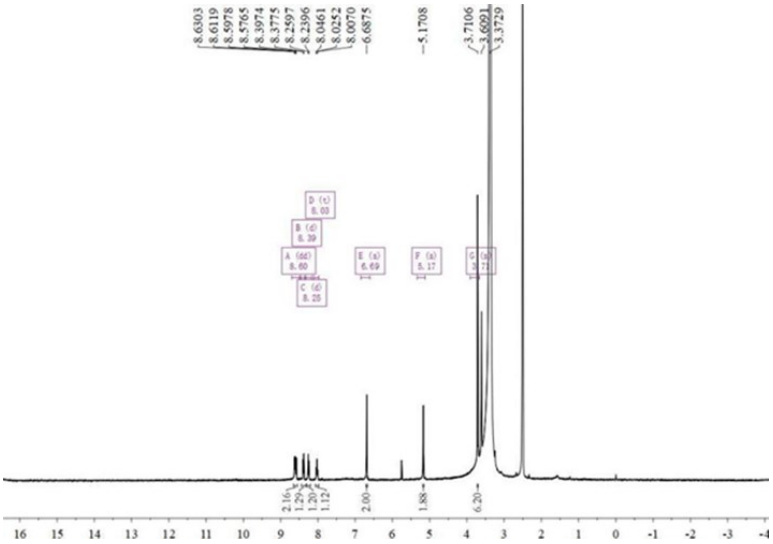

Fig. S4. ^{1}H NMR of GNTB

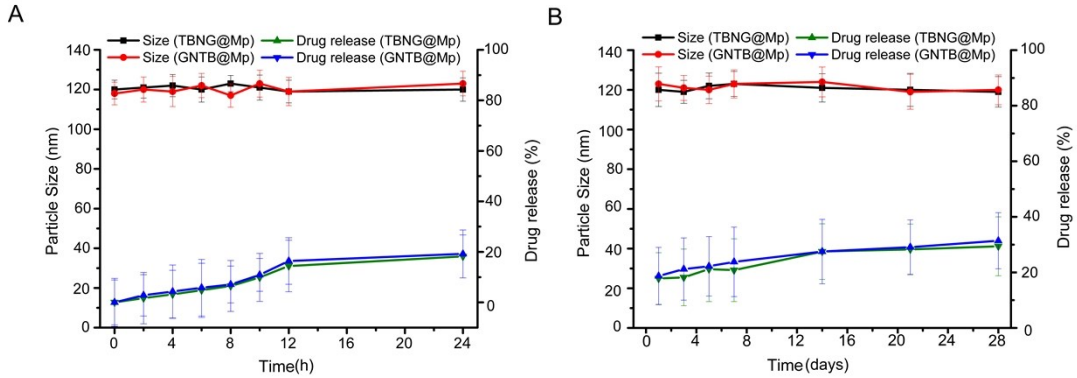

Fig. S5 Stabilities in serum (A) and the long-term stabilities (B) of TBNG@Mp and GNTB@Mp

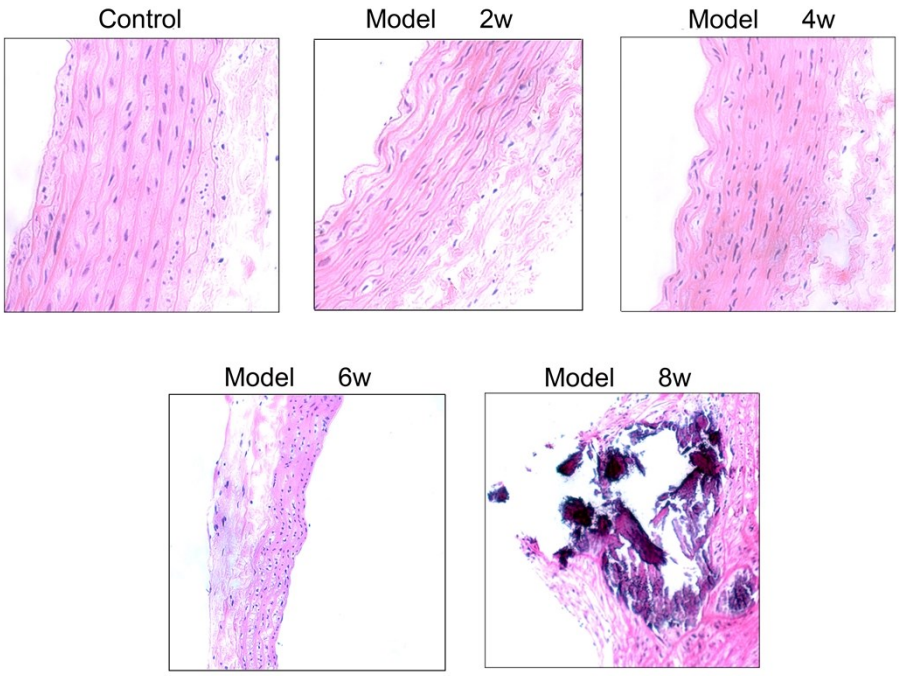

Fig. S6 H&E staining pictures of rat's thoracic aorta after high-fat feeding for different periods

3. TABLE

Table S1 Changes of rats' blood lipids in each group during modeling

Time (weeks)	Group	TG	TC	HDL-C	LDL-C
0	Control	1.10	1.67	1.15	0.23
	Model	0.25	7.13	0.73	4.32
		1.60	4.78	1.42	1.85
2	Control	1.21	1.54	1.06	0.15
	Model	0.39	5.83	1.08	3.21
		1.30	6.78	1.79	2.67
4	Control	1.04	1.73	1.19	0.17
	Model	0.22	5.40	1.09	2.90
		0.38	6.96	1.60	3.28
6	Control	1.33	1.38	1.21	0.24
	Model	1.55	4.63	1.93	1.62
		0.48	5.83	1.84	2.26
8	Control	1.16	1.42	1.35	0.19
	Model	0.65	4.47	1.43	2.32
		0.48	6.18	1.74	2.76