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Characterization and Biological Evaluation of Structurally Modified Taurine using Benzaldehydes

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Four Schiff base compounds (**1-4**) were synthesized by the reaction of taurine and benzaldehyde characterized by methoxy substitution at different positions. The chemical structures of these new compounds were determined using IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra. They are potassium 2-{[1-(3,5-dimethoxy-phenyl)meth-(Z)-ylidene]amino}ethane sulfonic acid, potassium 2-{[1-(3,4,5-trimethoxy-phenyl)-meth-(Z)-ylidene]amino}ethane sulfonic acid, potassium 2-{[1-(2,5-dimethoxy-phenyl)meth-(Z)-ylidene]amino}ethane sulfonic acid and potassium 2-{[1-(2,4,5-trimethoxy-phenyl)meth-(Z)-ylidene]amino}ethane sulfonic acid. The cytotoxicity of these taurine benzaldehyde Schiff bases and the effects of taurine benzaldehyde Schiff bases on the contractility of isolated jejunal segment (IJS) were determined. The results showed that no cytotoxic effects on caco-2 cell lines were observed when the concentrations of taurine benzaldehyde Schiff bases were below 50,000 μM and compounds **1**, **2** increased and compounds **3**, **4** decreased the contractility of isolated jejunal segment, indicating that methoxy substitution in different positions of taurine benzaldehyde Schiff bases may modify its effects on the contractility of isolated jejunal segment.

Key Words: Taurine, Schiff base compounds, Contractility.

INTRODUCTION

Compounds with the structure of AC=NB are known as Schiff bases, which arouse considerable interest due to their antifungal, anticancer and antibacterial activities[1-5]. The Schiff bases compounds can be synthesized from the condensation of primary amines and active carbonyl groups.

Taurine (2-aminoethane sulfonic acid), which belongs to an organic acid widely distributed in animal tissues, is a non-protein β-amino acid, playing an important role in many physiological processes *i.e.*, regulation of cardiovascular responses and neuronal excitability, maintenance of structure and function of photoreceptors, modulation of neurotransmitter and hormone release[6-9]. Taurine has primary amino groups on its side chain and is widely used as an organic ligand. Relevant documents have reported that taurine forms multiple types of Schiff base compounds[10-12]. However, these studies focus mainly on the synthesis, crystallization, antifungal, anticancer and antibacterial activities. Based on that taurine is found to modulate smooth muscle contraction[13-16], while the effect of Schiff base on contractility of isolated jejunal segment is rarely reported and our preliminary assay found that Schiff bases slightly inhibited the contractility of smooth muscle. These lead us to suspect the possible effect of taurine Schiff base on the contractility of smooth muscle.

The present study is designed to synthesize novel compounds with taurine and substituted benzaldehydes and to characterize their effects on the contractility of smooth muscle.

EXPERIMENTAL

Synthesis of the compounds (1-4): 10 mmol taurine and 10 mmol potassium hydroxide were dissolved in 20 mL distilled water. To this solution, 10 mmol 2,5-dimethoxy benzaldehyde, 3,5-dimethoxy benzaldehyde, 3,4,5-*tris*-methoxy benzaldehyde and 2,4,5-*tris*-methoxy benzaldehyde, which dissolved, respectively in 20 mL methanol solution were added dropwise within 10 min. The mixtures were stirred and heated at 50 ºC for 2 h, then cooled to room temperature. After filtration, the filtrate was left to stand at room temperature and evaporate naturally. The synthetics were then washed with methanol and dried in air to get target product. No benzaldehydes and taurine were then identified in the newly synthesized compounds.

Compound 1: Potassium 2-{[1-(3,5-dimethoxy-phenyl)-meth-(Z)-ylidene]amino}ethane sulfonic acid. White, yield = 86 %, m.p. 205-207 ºC. IR (KBr, ν_{max}, cm^{-1}): 1649 (C=N), 1192, 1155, 1034 (-SO_3). ^{1}H NMR (DMSO-d_6) δ_H: 8.29 (s, 1H; N=CH, H-7), 6.90 (2H, d, J = 2.4 Hz, H-2, 6), 6.57 (1H, d, J = 2.4 Hz, H-4), 3.82 (2H, m, H-9), 2.76 (2H, m, H-10),

3.77 (6H, s, 2 × -OCH_3). δ_C: 161.2 (N=CH, C-7), 105.5 (C-1), 152.8 (C-2), 160.6 (C-3), 102.8 (C-4), 160.6 (C-5), 105.5 (C-6), 57.2 (C-9), 52.3 (C-10). ESI-MS: $[M + H]^+$ m/z 313.4. Anal. calcd. (%) for $C_{11}H_{15}NO_5SK$: C: 42.29, H: 4.84, N: 4.48, S: 10.26. Found (%): C: 42.33, H: 4.81, N: 4.54, S: 10.32.

Compound 2: Potassium 2-{[1-(3,4,5-trimethoxy-phenyl)meth-(Z)-ylidene]amino}ethane sulfonic acid. White, yield = 91 %, m.p. 190-192 ºC. IR (KBr, ν_{max}, cm^{-1}): 1647 (C=N), 1206, 1173, 1057 (-SO_3). ^1H NMR (DMSO-d_6) δ_H: 8.28 (s, 1H; N=CH, H-7), 7.06 (2H, s, H-2, 6), 3.81 (2H, m, H-9), 2.75 (2H, m, H-10), 3.82 (6H, s, 2× -OCH_3), 3.69 (3H, s, -OCH_3). δ_C: 161.0 (N=CH, C-7), 105.0 (C-1), 105.0 (C-2), 153.1 (C-3), 152.2 (C-4), 153.1 (C-5), 105.0 (C-6), 57.2 (C-9), 52.4 (C-10). ESI-MS: $[M + H]^+$ m/z 343.4. Anal. calcd. (%) for $C_{12}H_{17}NO_6SK$: C: 42.09, H: 5.00, N: 4.09, S: 9.36. Found (%): C: 42.13, H: 5.08, N: 4.01, S: 9.32.

Compound 3: Potassium 2-{[1-(2,5-dimethoxy-phenyl)-meth-(Z) -ylidene]amino}ethane sulfonic acid. White, yield = 91 %, m.p. 173-175 ºC. IR (KBr, n_{max}, cm^{-1}): 1642.45 (C=N), 1200.86, 1171.91, 1035.90 (-SO_3). ^1H NMR (DMSO-d_6) δ_H: 8.62 (s, 1H; N=CH, H-7), 7.33 (1H, d, J = 2.8 Hz, H- 6), 7.02 (1H, m, H-3, 4), 3.83 (2H, m, H-9), 2.75 (2H, m, H-10), 3.80, 3.73 (6H, s; -OCH_3). δ_C: 156.2 (N=CH, C-7), 124.5 (C-1), 152.8 (C-2), 110.2 (C-3), 113.4 (C-4), 153.1 (C-5), 118.1 (C-6), 57.8 (C-9), 52.4 (C-10). ESI-MS: $[M + H]^+$ m/z 313.4. Anal. calcd. (%) for $C_{11}H_{15}NO_5SK$: C: 42.29, H: 4.84, N: 4.48, S: 10.26. Found (%): C: 42.33, H: 4.81, N: 4.54, S: 10.32.

Compound 4: Potassium 2-{[1-(2,4,5-trimethoxy-phenyl)-meth-(Z)-ylidene]amino}ethane sulfonic acid. White, yield = 91 %, m.p. 190-192 ºC. IR (KBr, ν_{max}, cm^{-1}): 1634.14 (C=N), 1219.95, 1128.24, 1041.35 (-SO_3). ^1H NMR (DMSO-d_6) δ_H: 8.55(s, 1H; N=CH, H-7), 6.72(1H, s, H-3), 7.33 (1H, s, H-6), 3.77 (2H, m, H-9), 2.74 (2H, m, H-10), 3.84 (6H, s, 2× -OCH_3), 3.71 (3H, s, -OCH_3). δ_C: 155.7 (N=CH, C-7), 115.5 (C-1), 153.8 (C-2), 97.7 (C-3), 152.2 (C-4), 143.0 (C-5), 108.8 (C-6), 57.7 (C-9), 52.6 (C-10). ESI-MS: $[M + H]^+$ m/z 343.4. Anal. calcd. (%) for $C_{12}H_{17}NO_6SK$: C: 42.09, H: 5.00, N: 4.09, S: 9.36. Found (%): C: 42.11, H: 4.97, N: 4.12, S: 9.39.

Structure determination: IR spectra were determined using IR 200 spectrometer (Thermo Electron Corporation). ^1H and ^{13}C NMR spectra were measured on a JNM-LA-500 spectrometer with tetramethylsilane (TMS) as an internal standard and chemical shifts were recorded in δ ppm. ESI-MS was measured on a Finnigan LCQ LC-MS spectrometer.

Cytotoxic activities: Cytotoxicity was assessed by using the standard MTT analysis[17,18]. Caco-2 Cell suspension (1 × 10^5 cells/mL) were subdivided and placed in a 96-well culture plate, 100 µL per well. Cells were incubated at 37 ºC in 5 % CO_2 for 24 h. Then 90 µL new Dulbecco's Modified Eagle Medium (DMEM) and 10 µL serial dilutions of compounds **1-4** were added into well, including control wells of DMEM and cell alone without taurine benzaldehyde Schiff base (TBSB) compounds. Each assay was repeated for three times. The contents were incubated in a 5% CO_2 - buffered and humidified incubator at 37°C for 20 h and left for further 4 h after 10 µL MTT reagent was added to each well. 100 µL detergent reagent was added to each well and the culture plates were incubated overnight.

The optical density (OD) was recorded using a microtiter plate reader at 570 nm. Cell proliferation in the presence of compounds **1-4** exposure was determined as percentage viability of the control:

$$\text{Viability} = \left(\frac{OD_C}{OD_N}\right) \times 100\ \%$$

OD_C is the OD value of the compounds **1-4** treated cells; OD_N is the OD value of the control (no compound **1-4**) cells.

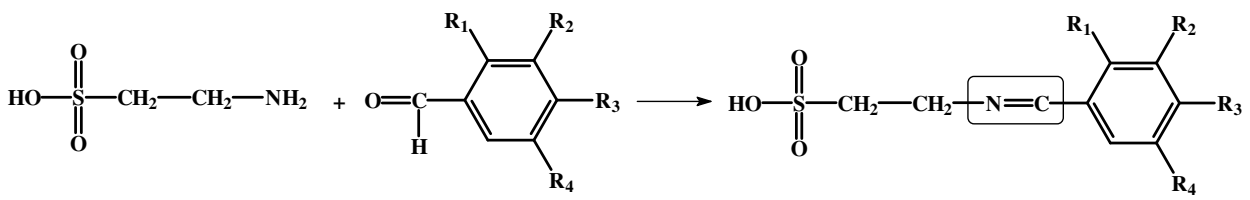
Determination of isolated jejunal segment contractility: All rats were treated according to the guidelines for the Care and Use of Laboratory Animals of Dalian Medical University and all experimental procedures described were carried out in accordance with the Declaration of Helsinki. Male Sprague-Dawley rats, weighted 180-200 g, were fasted for 24 h but water before experiments. For jejunal contractility measurement, rats were sacrificed by cervical dislocation and the jejunum was gently removed and then kept in Krebs buffer.

The jejunum was rinsed and clipped into small segments (*ca.* 12 mm long). The segment was suspended in longitudinal direction in a 20 mL organ baths with warmed (37 ºC) and aerated Krebs buffer. One gram resting tension was applied to each 1 cm segment that was equilibrated for 1 h. The contractile responses of JLS were recorded in a BL-420 physiological recording system.

RESULTS AND DISCUSSION

Four taurine benzaldehyde Schiff base were synthesized by using the methods described in **Scheme-I**. The chemical structures of the taurine benzaldehyde Schiff base were determined by using IR, ^1H NMR, ^{13}C NMR, ESI-MS spectra and elemental analysis.

IR spectra of the taurine benzaldehyde Schiff base showed characteristic sulfonic acid absorption and confirmed sulfonic acid participated in the coordination. The disappearance of 3500-3300 cm^{-1} absorption indicated the formation of taurine benzaldehyde Schiff base. The carbonyl absorption in methoxy



Compound 1: $R_{2,4}$ = OCH_3; $R_{1,3}$ = H **Compound 2: $R_{2,3,4}$ = OCH_3; R_1 = H**
Compound 3: $R_{1,4}$ = OCH_3; $R_{2,3}$ = H **Compound 4: $R_{1,3,4}$ = OCH_3; R_2 = H**

Scheme-I: Synthetic procedure and characterizations of compounds **1-4**

substituted benzaldehyde shifted towards 1649-1634 cm^{-1} due to C=N stretching in new Schiff bases, confirmed by ^{13}C NMR (DMSO-d_6, 100 MHz), showing CH=N at δ 155.7-161.2 (C-7). Whereas in the ^{1}H NMR (DMSO-d_6, 400 MHz), the CH=N protons of compounds **1-4** appeared in δ 8.28-8.62 as a singlet. The data showed that the Schiff base fragments existed in the structure of synthetic, indicating it was the target product.

Using MTT assay[17,18], all the taurine benzaldehyde Schiff base synthesized were tested for the cytotoxicity against Caco-2 cells which could represent for epidermal cells of normal small intestine. No cytotoxic effects on Caco-2 cell lines were observed when the concentration of taurine benzaldehyde Schiff base was below 50000 μM (Fig. 1). The synthesized taurine benzaldehyde Schiff base in the concentration of 50-800 μM were used to determine its effects on the contractility of isolated jejunal segment.

The newly synthesized taurine benzaldehyde Schiff base were tested on the contractility of isolated jejunal segment using taurine as the control. In the same concentration range of 50-800 μM, the effects of taurine benzaldehyde Schiff base on the contractility of isolated jejunal segment were as follows

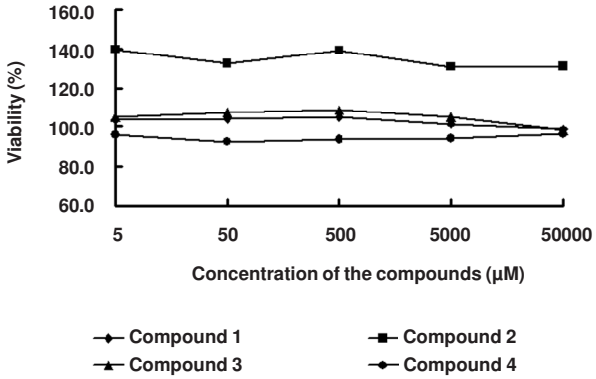
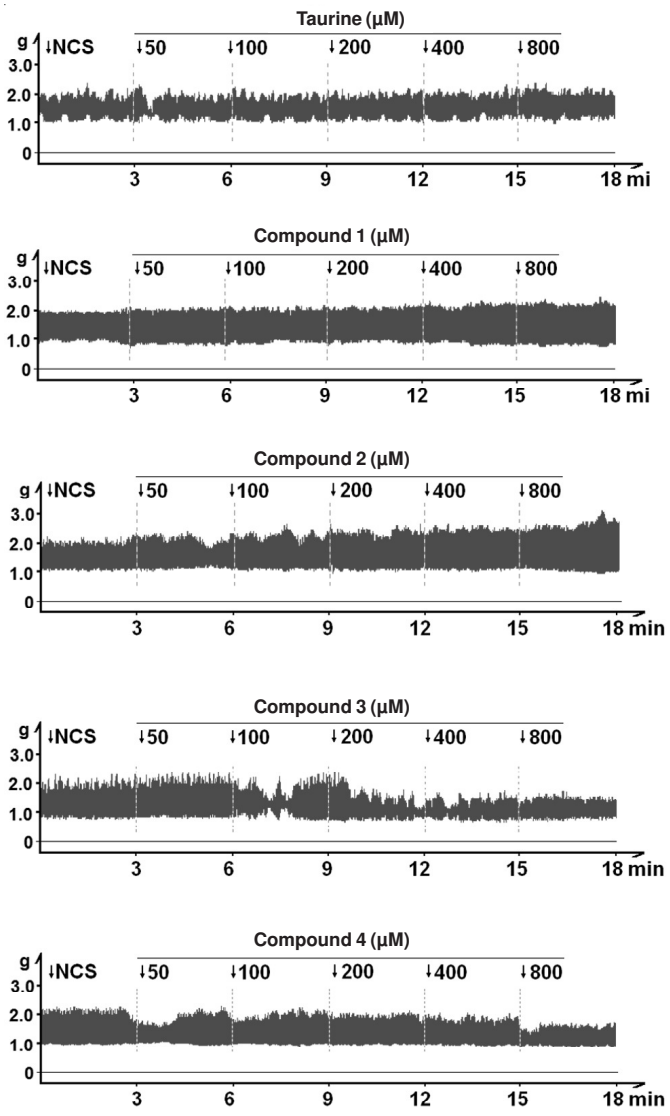


Fig. 1. Cytotoxicity of the compound **1-4** against caco-2 cells

(Fig. 2). Compounds **1**, **2** significantly increased the contractility of isolated jejunal segment in a dose dependent manner. However, compound **3** and **4** significantly decreased contractility of isolated jejunal segment ($p < 0.05$, Fig. 2) in a dose dependent manner.

The biological activity of a particular substance depends on a complex sum of individual properties including compound structure, affinity for the target site, survival in the medium of



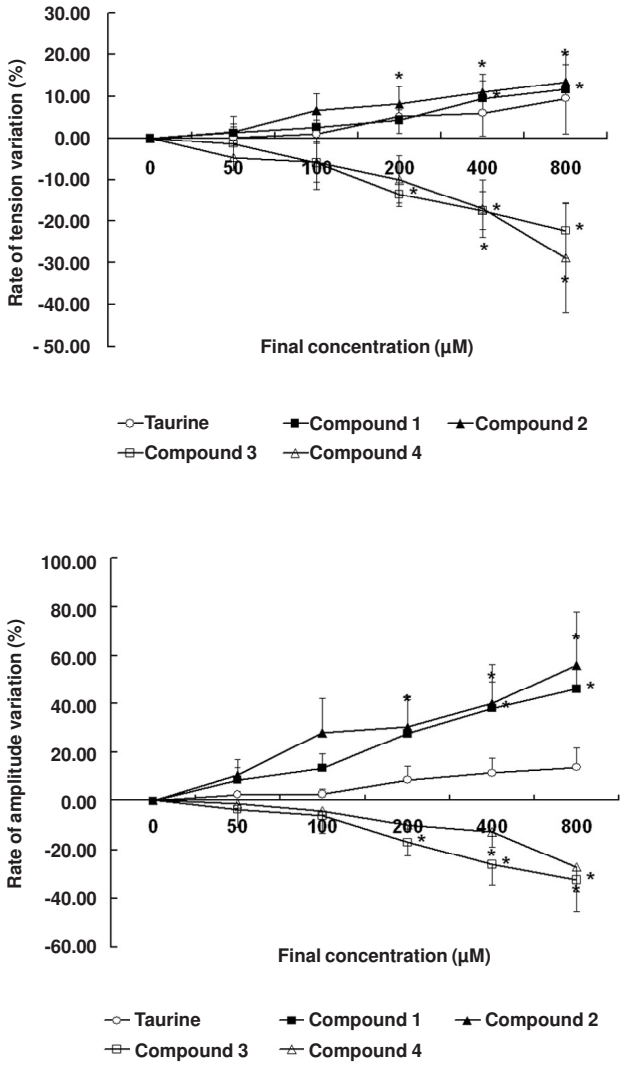


Fig. 2. Effect of compounds **1-4** on the contractility of IJS

application, survival within the biological system, transport properties and state of the target organism[19]. Based on that compound **1** and **2** showed stimulatory and compound **3** and **4** showed inhibitory effects on the contractility of isolated jejunal segment, the characterization of the structure-activity relationship of the four compounds is as follows. Compound **1** has H substitution at position 4 and compound **2** has methoxy substitution at position 4. However, they produce similar stimulatory effects, implicating position 4 is not critical for producing stimulatory effects. Compound **3** has H substitution at position 5 and compound **4** has methoxy substitution at position 5. However, compounds **3** and **4** have similar inhibitory effects, implicating position 5 is not critical for producing inhibitory effects. We noted that compound **1** and compound **2** have the identical methoxy substitution occurring at positions 3 and 5 and they have same stimulatory effects, implicating that methoxy substitution at positions 3 and 5 is primary for producing stimulatory effects; compound **3** and compound **4** have the identical methoxy substitution at positions 2 and 4 and they have same inhibitory effects, showing that methoxy substitution at positions 2 and 4 is primary for producing inhibitory effects.

Conclusion

Present results showed that the novel taurine benzaldehyde Schiff base possessed different effects on the contractility of isolated jejunal segment. The stimulatory and inhibitory effects of the compounds are found to be correlated with the methoxy substitution at the different position of the Schiff base. Further study is needed for preclinical evaluations of taurine benzaldehyde Schiff base in relieving intestinal abnormal contractility, for instance, irritable bowel syndrome.

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