A target lipidomics approach to investigate the acute inflammatory irritation induced by indolealkylamines from Chansu water fraction in rats

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[ABSTRACT] Chansu has demonstrated adverse reactions in clinical settings, which is associated with its toxicity and limits its clinical applications. But there are methodological limitations for drug safety evaluation. In the current study, ultra-high performance liquid chromatography, lipidomic profiling, and molecular docking were used to systemically assess Chansu-induced acute inflammatory irritation and further identify the underlying drug targets. Compared with the EtOAc extract, Chansu water fraction containing indolealkylamines caused acute inflammatory irritation in rats, including acute pain (spontaneous raising foot reaction), and inflammation (paw edema). At the molecular level, lipids analysis revealed significantly higher levels of pro-inflammatory mediators of the COX and LOX pathways. However, anti-inflammatory mediators from the CYP 450, ALA, and DHA pathways markedly decreased after exposure to Chansu water fraction. Moreover, four indolealkylamines from Chansu showed a high theoretical affinity to a known irritation target, 5-HT2A R. These results suggest that Chansu-induced inflammatory irritation is related to the distinct dysregulation of inflammatory lipids, and peripheral 5-HT2A R is a potential target for irritation therapy. The strategy used in this study can be a crucial approach in the safety evaluation of natural medicinal substances.

[KEY WORDS] Chansu; Acute inflammatory irritation; Lipidomics; Eicosanoids; Molecular docking


Introduction

Adverse drug reaction (ADR) is defined as an appreciably harmful or unpleasant reaction resulting from an intervention related to the use of a medicinal product [1, 3], including side effects, toxic reactions, allergic reactions, etc. However, the classical toxicological methods for investigating drug safety and mechanisms of action in animal studies are inefficient and insufficient [3]. Acute toxicity and long-term toxicity of new drugs require more time and preclinical testing in animals. Moreover, there is a significant challenge in predicting local tissue damage. Therefore, a precise, rapid, and high-throughput method to evaluate the safety of drugs is needed.

Chansu, referred to as “toad venom”, is a well-known traditional Chinese medicine, which has been widely used as a cardiotoxic and antineoplastic agent in Asia [4]. In clinical practice, Chansu and some popular preparations (e.g., Chansu injection, Liushenwan, and Shexiangbaoxindan) are known to cause obvious side effects. Traditionally, many researches mainly focused on the cardiotoxicity induced by lipophilic bufadienolides from Chansu [1, 4]. For indolealkylamines, hydrophilic constituents existing in Chansu include serotonin, N-methyl serotonin, bufotenedine, and bufotenidine [5]. They were also reported to cause severe adverse reactions, such as vaso-
Lipidomics, which is an area of study dedicated to comprehensive analysis and characterization of the functions and metabolism of lipids in biological samples, has recently shown potentials in the identification of potential biomarkers for safety evaluation [9]. Eicosanoids, one of the most comprehensively studied classes of lipids, play an essential role in the regulation of inflammatory and immune functions [10]. Recently, the metabolism of nine lipids have been monitored in a metabolomics study of Chansu-induced acute toxicity in rats [11]. In our previous study, Chansu water fraction caused a dose-dependent inflammatory irritation [12]. However, the related constituents and mechanisms of Chansu-induced irritation have not been adequately elucidated. In the current study, the chemical constituents of Chansu extracts were analyzed by ultra-high performance liquid chromatography, and their noxious responses and eicosanoid-related biomarkers were evaluated in rats. Furthermore, molecular docking was used to characterize the binding between indolealkylamines from Chansu and 5-HT₃ receptors.

Materials and Methods

Sample preparation
Chansu was purchased from Nanjing Medicinal Material Company (Jiangsu, China) and authenticated by Professor DUAN Jin-Ao (Nanjing University of Chinese Medicine). Chansu powder (40 mg) was separately dissolved in 1 mL 0.5% DMSO and ethyl acetate (EtOAc) before ultrasound treatment for 20 min twice. The extracts were collected by centrifugation at 12000 r·min⁻¹ for 30 min, concentrated, and sufficiently dried for later use. Two different solvent extracts were redissolved in 2 mL 0.5% DMSO to a final crude concentration of 20 mg mL⁻¹, and used for animal experiments.

Chemical composition analysis
UHPLC analysis of the water-soluble fraction of Chansu was performed using an Agilent 1290 Infinity Liquid Chromatography system with a Durashell RP C₁₈ column (4.6 mm × 250 mm, 5 μm). The mobile phase consisted of A (10 mmol·L⁻¹ ammonium acetate aqueous solution) and B (acetoneitrile) using a linear gradient elution as follows: 0–12 min, 4% B; 12–20 min, 4%–5% B; 20–25 min, 5%–6% B. The flow rate was 1 mL·min⁻¹, the UV detection wavelength was 275 nm, and the injection volume was 3 μL.

UHPLC analysis of Chansu EtOAc extract was performed on a Kromasil C₁₈ column (4.6 mm × 100 mm, 3.5 μm). Mobile phases A (0.1% formic acid aqueous solution) and B (acetonitrile) were used for gradient elution. The elution gradient was listed as follows: 0–6 min, 20%–25% B; 6–12 min, 25%–30% B; 12–24 min, 30%–35% B; 24–34 min, 35%–40% B; 34–35 min, 40%–90% B; 35–37 min, 90% B; 37–38 min, 90%–20% B; 38–42 min, 20% B. The flow rate was 0.7 mL·min⁻¹, the injection volume was 5 μL, and the UV detection wavelength was 296 nm.

Paw inflammatory irritation experiment in rats
Male Sprague-Dawley rats (180–220 g) were obtained from the Experimental Animal Center of Zhejiang (Zhejiang, China, animal certificate number SCXK-zhe-2014-0001). The rats were housed under controlled environmental conditions at a stable temperature (23 ± 3 °C) and humidity (60% ± 5%) with free access to food and water. Animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Research Council of USA, 1996) and related ethical regulations of Nanjing University of Chinese Medicine.

Chansu-induced inflammatory irritation in rats was performed according to our previous method [12]. Rats were randomly divided into three groups. Group 1 (Normal) received normal saline as control; group 2 (Chansu-Water) received Chansu 0.5% DMSO extract; and group 3 (Chansu-EtOAc) received Chansu EtOAc extract. The animals were intraperitoneally administered with sample solutions (100 μL/paw) under the mid-planter surface of the right hind paw. The total number of times of raising the foot were counted between 0 and 10 min after drug administration. Furthermore, injected paw edema was measured before experiment and 30 min after injection of sample solution.

Lipidomics analysis
Frozen paw samples were obtained from our previous study [12]. The rats in the experimental groups were given 10 mg·mL⁻¹ and 50 mg·mL⁻¹ Chansu water fractions, respectively. All samples were allowed to thaw on ice. Liquid-liquid extraction was used to separate lipid mediators from the paw. Ethyl acetate and n-hexane (V/V = 1 : 1, cooled to −80 °C) were added to 10% paw homogenates with a mass and volume ratio of 1 : 8. The sample was vortexed, ultrasonicated at 100 Hz for 20 min in an ice water bath, and centrifuged at 2000 rpm·min⁻¹ for 5 min. The supernatants were transferred to a fresh tube, concentrated using SpeedVac, and re-constituted in 500 μL of 90% acetonitrile solution containing chloramphenicol (200 ng·mL⁻¹) for LC-MS/MS analysis [13].

Chromatography was performed on a Synergi RP C₁₈ column (2.0 mm × 50 mm, 4 μm) using the UHPLC system (SHIMADZU LC-20AD XR). The column was maintained at 35 °C and the flow rate was 0.30 mL·min⁻¹. The mobile phase was composed of solvent A (water-acetonitrile-formic acid = 70 : 30 : 0.02, V/V/V) and solvent B (acetonitrile-isopropanol alcohol = 50 : 50, V/V). The samples were separated as follows: 0–3 min, 0%–25% B; 3–11 min, 25%–45% B; 11–13 min, 45%–60% B; 13–18 min, 60%–75% B; 18–18.5 min, 90% B; 18.5–20 min, 90% B; 20–21 min, 90%–0% B; 21–25 min, 0% B. The injection was 5 μL. The UHPLC was directly interfaced with an AB Sciex QTRAP 5500 system (AB SCIEX, Foster City, CA, USA) spectrometer with an ESI source operated in the negative ion mode. Parameters in the source were set as follows: ion spray voltage, −4500 V; turbo ion spray temperature, 525 °C; curtain gas, 10 psi; nebulizer gas (GS 1), 30 psi; heater gas (GS 2), 30 psi; and dwell time, 50 ms. Due to the large amount of lipids to be determined in one sample, each sample was detected 3 times by
inserting different ion pairs using LC-MS/MS. Data were collected in the multiple reaction monitoring (MRM) mode. Chloramphenicol as the internal standard was used for quality control. The data of the lipid metabolites were transferred to SIMCA-P software for principal component analysis (PCA), and orthogonal projections to latent structures discriminant analysis (OPLS-DA).

**Molecular docking analysis**

The complex crystal structures of indolealkylamines from Chansu and 5-HT$_2$A R (Protein Data Bank identification code: 6A94), were used to generate the acceptor model for docking experiments. The three-dimensional structural information was downloaded from the PubChem Public Chemical Database. Molegro Virtual Docker software (MVD) (http://www.molegro.com/products.php), a precise semi-flexible molecular docking program was used for docking analysis. By increasing the qualifications, the recognition accuracy of bonding models was enhanced. Compared with other molecular docking software, MVD yielded higher accuracy (MVD: 87%, Glide: 82%, Surfex: 75%, FlexX: 58%) [14].

Using the MolDock evolution algorithm, MVD predicted the interaction between the ligand and 5-HT$_2$A R protein, as well as the intra-molecular interaction energy of the ligand. Before screening, a docking protocol was validated. Generally, 10 to 100 individual docking simulations were performed. Each possible docking model was analyzed according to the MolDock scores, Rerank scores, Hydrogen bonds, and known key amino acid residues in the active site.

**Data analysis**

Data are expressed as mean ± SD Statistical analysis was performed using a two-tailed unpaired Student’s t-test. A *P* value of less than 0.05 was considered statistically significant, while a *P* value less than 0.01 was very significant. All statistical analyses were conducted using Microsoft Excel and performed by GraphPad Prism 7.0 software.

**Results**

**UHPLC-UV analysis of Chansu extracts**

Indolealkylamines such as serotonin, bufotenidine, and bufotenine were the main components of Chansu water fraction (Fig. 1). Bufadienolides were the major chemical constituents in Chansu EtOAc extract (Supplementary Fig. 1). The total mass of the four alkaloids was 4.95% of Chansu, serotonin, bufotenidine, bufotenine, and bufothionine accounted for 1.43%, 2.60%, 0.87%, and 0.05% of Chansu, respectively. Among them, serotonin and bufotenidine accounted for 4.03% of Chansu and 81.47% of the four alkaloids. The chromatograms of Chansu water fraction and mixed standards were shown in Fig. 2.

**Paw inflammatory irritation in rats**

The irritant effects of Chansu extracts were evaluated using a paw inflammatory irritation model. As shown in Fig. 3A, the number of times of raising the foot significantly increased within 10 min after administration of Chansu water fraction (*P* < 0.01). However, such a significant increase was not observed in the Chansu-EtOAc group. The number of times of raising the foot in the Chansu-water and Chansu-EtOAc groups was 93 ± 10 and 3 ± 1, respectively. Paw edema induced by Chansu water fraction (0.128 ± 0.015 mL) was also obvious compared with the normal group (0.067 ± 0.017 mL) (*P* < 0.05). The paw edema of the Chansu-EtOAc group was 0.091 ± 0.011 mL (Fig. 3B). These results showed that Chansu water fraction caused obvious acute inflammatory irritation in rats.

**Determination of lipid metabolites**

To better characterize the inflammation of paw tissues, a target lipidomics approach was used [15]. A total of 121 inflammatory lipids were detected based on different retention times and accurate masses. These inflammatory lipids included lipids generated through the cyclooxygenase (COX), lipoxigenase (LOX), and cytochrome P450 (CYP 450) pathways derived from arachidonic acid, as well as metabolites derived from linoleic acid (LA), α-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), and others. Among them, 80 core lipid mediators were semi-quantified using chloramphenicol as the internal standard were shown in Fig. 2.

![Fig. 2](image-url)  The representative chromatograms of four mixed standard compounds and Chansu water fraction analyzed by UHPLC. (A) Four standard compounds and (B) Chansu water fraction. (1) serotonin; (2) bufotenidine; (3) bufotenine; and (4) bufothionine.
Effects of Chansu water fraction on eicosanoid biomarkers

The relative contents of lipid metabolites between the Chansu water fraction and normal groups were different (Fig. 6). In the PCA model, all samples were clustered into three groups ($R^2_X = 0.888$, $Q^2 = 0.753$) and colored according to different dosage regimens (Fig. 7A). OPLS-DA was performed to determine the predictive variables responsible for inter-group differences. From the OPLS-DA score plot, three experimental groups showed an obvious clustering trend ($R^2 = 0.997$, $Q^2 = 0.880$) (Fig. 7B). Therefore, lipids with variable importance in the projection (VIP) > 1 and more than a 1.5-fold change (FC > 1.5) in content were selected as biomarkers (Fig. 7C) [17]. These potential biomarkers may be responsible for Chansu-induced paw inflammation in rats.

Specifically, there were 14 up-regulated (FC > 1.5) lipids after treatment with Chansu water fraction, among which nine eicosanoids from the COX pathway significantly increased ($P < 0.05$), including PGJ$_2$, PGF$_{2\alpha}$, PGE$_2$, and 8-iso-PGF$_{2\alpha}$ etc. (Fig. 6 and Fig. 8). For LOX products, only LTC$_4$ significantly increased in the Chansu-W 10 mg·mL$^{-1}$ group ($P < 0.05$) (Fig. 8). The tetranor 12-HETE and 15-HpETE derived from the LOX pathway were up-regulated (FC > 1.5); however, there was no significant difference between the normal and Chansu water fraction groups ($P > 0.05$) (Fig. 8). The 11-HEDE produced from the LA pathway and EPA in the EPA pathway also had more than a 1.5-fold increase ($P < 0.05$) after administration of Chansu water fraction. Besides, some lipids markedly decreased ($P < 0.05$) in the Chansu-W groups, including 8, 9-DiHETeR and 5, 6-DHET produced from the CYP pathway, 13-oxoODE from the LA pathway, DHA, 16-HDoHE, and NPD1 from the DHA pathway (Fig. 8 and Fig. 9). Other mediators from the LOX, EPA, and other pathways were not distinctly affected in rats treated with Chansu water fraction.

Analysis of pro- and anti-inflammatory mediators

To determine the changes of inflammatory mediators in rats, the lipids were grouped based on their pro- or anti-inflammatory properties. 12 Pro-inflammatory mediators and 20 anti-inflammatory mediators were screened (Fig. 10A). Results showed up-regulation of pro-inflammatory mediators of the COX and LOX pathways, and down-regulation of anti-inflammatory mediators from the CYP 450, ALA, and DHA pathways in the Chansu-W groups. The significant up-regulation of pro-inflammatory mediators after administration of Chansu water fraction ($P < 0.01$), was associated with high levels of lipids such as PGF$_{2\alpha}$, 5,6-DHET, etc (Fig. 6 and Fig. 8). For LOX products, only LTC$_4$ increased ($P < 0.05$) after administration of Chansu water fraction. Besides, some lipids markedly decreased ($P < 0.05$) in the Chansu-W groups, including 8, 9-DiHETeR and 5, 6-DHET produced from the CYP pathway, 13-oxoODE from the LA pathway, DHA, 16-HDoHE, and NPD1 from the DHA pathway (Fig. 8 and Fig. 9). Other mediators from the LOX, EPA, and other pathways were not distinctly affected in rats treated with Chansu water fraction.

Table 1 Molecular docking of the four alkaloids from Chansu water fraction binding to 5-HT$_{2A}$R

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MolDock score (KJ/mol)</th>
<th>Rerank score (KJ/mol)</th>
<th>H-bond score</th>
<th>H-bond receptor of ligand (energy score)</th>
<th>Interaction residues within 5-HT$_{2A}$R (energy score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td>101.08</td>
<td>82.52</td>
<td>−4.30</td>
<td>Val 156 (−0.10), Thr 160 (−2.38), Asp 155 (−0.82), Ser 159 (−2.25), Asp 155 (−2.50)</td>
<td>Asp 155 (−9.64), Ser 159 (−4.31), Thr 160 (−3.54), Ser 242 (−4.31), Trp 336 (−2.40)</td>
</tr>
<tr>
<td>Bufotenidine</td>
<td>116.51</td>
<td>95.17</td>
<td>−2.50</td>
<td>Val 156 (−0.02), Thr 160 (−2.48)</td>
<td>Asp 155 (−9.64), Ser 159 (−4.31), Thr 160 (−3.54), Ser 242 (−4.31), Trp 336 (−2.40)</td>
</tr>
<tr>
<td>Bufotenine</td>
<td>109.31</td>
<td>87.19</td>
<td>−1.66</td>
<td>Thr 160 (−1.66)</td>
<td>Asp 155 (−9.64), Ser 159 (−4.31), Thr 160 (−3.54), Ser 242 (−4.31), Trp 336 (−2.40)</td>
</tr>
<tr>
<td>Bufothionine</td>
<td>101.06</td>
<td>78.76</td>
<td>−3.40</td>
<td>Ser 242 (−2.50), Thr 160 (−0.90)</td>
<td>Asp 155 (−9.64), Gly 238 (−2.00), Ser 242 (−4.31), Trp 336 (−2.40), Ser 159 (−4.31), Thr 160 (−3.54)</td>
</tr>
</tbody>
</table>
Inflammatory effects, were present at much lower levels in Chansu treated animals (Fig. 10A), and an overall significant decrease was observed in the Chansu-W groups \((P < 0.01)\) compared with the normal group (Figs. 10C, 10D).

Molecular docking of indolealkylamines/5-HT\(_{2A}\)R

Molecular docking results were presented in Fig. 11, which illustrated that indolealkylamines were located at 5-HT\(_{2A}\)R cavity 1 (site 1) with the highest volume (Supplementary Fig. 2). The MolDock scores were determined as follows: bufotenidine \((-116.51 \text{ KJ/mol}) < \text{bufotenine} \ (-109.31 \text{ KJ/mol}) < \text{serotonin} \ (-101.08 \text{ KJ/mol}) < \text{bufothionine} \ (-101.06 \text{ KJ/mol})\) (Table 1), all of which were less than \(-100 \text{ KJ/mol}\) and showed very high interaction energy. These findings were important for analysis of the indolealkylamine/5-HT\(_{2A}\)R interaction mode.

Docking analysis demonstrated that four ligands were bound to 5-HT\(_{2A}\)R at two different regions of the receptor’s pocket. The first binding region at the pocket entrance was

Fig. 4  Total ion chromatograms (TICs) of a rat right hindpaw sample after injection of 50 mg·mL\(^{-1}\) Chansu water fraction for three times detected by LC-MS/MS
formed by Ser 242, Thr 160, Val 156, Ser 159, and Asp 155. Meanwhile, a bottom hydrophobic cleft in the ligand-binding pocket was surrounded by highly conserved aromatic or hydrophobic residues (consisting of Phe 340, Phe 332, and Trp 336). These key residues made several specific H-bond interactions with 5-HT$_{2A}$R site 1 compounds.

Among the four investigated alkaloids, bufotenidine exhibited the best ligand/protein interaction with 5-HT$_{2A}$R (−116.51 KJ/mol) (Table 1). Serotonin generated five H-bond interactions with surrounding amino acid residues Val 156, Thr 160, Asp 155, Ser 159, and Asp 155 (Table 1). Docking studies of bufotenine and bufothionine with 5-HT$_{2A}$R also exhibited similar docking scores and interaction residues. Importantly, four ligands were found to bind with 5-HT$_{2A}$R by forming H-bonds with Thr 160 and interacting with similar surrounding amino acid residues such as Asp 155, Ser 159, and others (Fig. 11 and Table 1). Therefore, virtual screening revealed that the four indolealkylamines from Chansu had a stronger binding affinity for 5-HT$_{2A}$R.

**Discussion**

According to recent reports, Chansu has attracted considerable interest as a promising potent drug for the prevention and treatment of cancer, but the potential side effects...
limit its efficacy. In the present study, we investigated Chansu-induced acute inflammatory irritation in rats using lipidomics and molecular docking. Our results revealed that Chansu water fraction containing indolealkylamines was the
When acute inflammation occurs, PGE$_2$ is abundant PG, and can induce fever, inflammation and pain. It is one of the most physiologically pain, and immune function in the body. Prostaglandins (PGs) derived from the COX pathway have strong pro-inflammatory effects. PGE$_2$ is one of the most physiologically abundant PGs, and can induce fever, inflammation and pain. When acute inflammation occurs, PGE$_2$ spontaneously gathers in the injured tissue areas, where it acts alone or synergistically with other mediators to induce inflammation. PGE$_2$, a known pro-inflammatory mediator, can participate in inflammation and pain, which is related to the activation of NF-xB and the expression of COX-2. For the LOX pathway, it also contains crucial mediators for inflammation development. LTB$_4$ and TCB$_4$ from the LOX pathway have shown to be involved in pro-inflammation progression. LTB$_4$ can promote neovascularization and macrophage recruitment in murine wet-type AMD models. LTC$_4$ is an important inflammatory mediator in the pathogenesis of asthma. Our results demonstrated that administration of Chansu water fraction induced the increase of COX-derived PGs and LOX-derived LTs, suggesting that these pro-inflammatory mediators play a role as leading candidate biomarkers for Chansu-induced irritation.

In contrast, some anti-inflammatory mediators from the CYP 450, ALA, and DHA pathways in the Chansu-W groups were down-regulated compared with the normal group. Cytochrome P450 enzymes can metabolize arachidonic acid to produce 16-, 17-, 18-, 19-, and 20-HETEs and 5-, 6-, 8-, 9-, 11-, 12-, 14-, and 15-EETs. The EETs provide protection by regulating platelet aggregation, vascular tone, and vascular smooth muscle cell activation. 5,6-8-DHTL, a stable metabolite of arachidonic acid, is a potential EDHF that mediates microvascular dilation. Cytochrome P450 enzymes can also metabolize linoleic acid to produce 9, 10- or 12, 13-EpOME, and sEH further convert them to 9, 10- or 12, 13-diHOME. In recent years, DHA metabolites have been shown to promote the resolution of inflammation.

Serotonin and its derivatives, the indolealkylamines from

![Fig. 7 Multivariate statistical evaluation of 80 core eicosanoids-related biomarkers. (A) The score plot of unsupervised PCA method, (B) the score plot of supervised OPLS-DA method, and (C) the VIP plot of supervised OPLS-DA method](image-url)
Chansu, are important endogenous signal molecules in the body and act on serotonin receptors. It is well known that the serotonergic system exerts inhibitory and facilitatory influences on the neural systems involved in pain processing and modulation, which depend on the sites of action, cell types, receptor subtypes, and pain condition. In the peripheral nervous system, 5-HT is considered an inflammatory mediator and can activate the nociceptor and advance the traumatic information transmission when administered by intraperitoneal and subcutaneous injection. Receptors in-

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**Fig. 8** Effect of Chansu water fraction on lipid mediators from the COX, LOX, and CYP 450 pathways in the hind paws of rats. Data are expressed as mean ± SD, n = 5. *P < 0.05, **P < 0.01 vs normal group.
In conclusion, this study demonstrated that Chansu water fraction can induce acute inflammatory irritation in rats using the paw inflammatory irritation experiment, which may be related to lipid metabolism disorders and activation of peripheral 5-HT_{2A}R by indolealkylamines. However, a more comprehensive safety evaluation of Chansu is required to provide systematic toxicological information. Overall, the findings of this study provide constructive evidence for the safe application of Chansu in clinical practice.

**Supplementary materials**

Supplementary information can be acquired by e-mail
Fig. 10  Pro- and anti-inflammatory mediators of lipidomic profiles in the normal and Chansu water fraction groups. (A) Heat maps of pro- and anti-inflammatory mediators in the Chansu water fraction groups. (B) Column vertical scatter plot of the percentages of pro-inflammatory mediators for individual animal samples. (C) Column vertical scatter plot of the percentages of anti-inflammatory mediators for individual animal samples. (D) Stacked bar graph of the percentages of pro- and anti-inflammatory mediators. *P < 0.05, **P < 0.01 vs normal group

Fig. 11  Molecular docking model (A1-D1) and interaction residues (A2-D2) of serotonin (A), bufotenidine (B), bufotene (C), andbufothionine (D) binding to 5-HT2A R. Green dotted lines indicated the hydrogen bonds (H-bonds) and key interaction amino acid residues (with energy scores) were shown as sticks that were color-coded by hydropathy type.
to corresponding author.

References


