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Research Article

Identification and Characterization of Bioactive Compounds Targeting Uropathogenic *Escherichia coli* from Sanjin Tablets

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Sanjin Tablets are completely natural preparation with significant efficacy in treating urinary tract infection. To identify the bioactive compounds from Sanjin Tablets, we separated components capable of binding to the soluble proteins of uropathogenic *Escherichia coli* (UPEC) by affinity binding and characterized their identities using liquid chromatography-mass spectrometry (LC-MS) analysis. Our study discovered eight compounds with *E. coli* protein-binding capabilities, and all these compounds were tracked back to the original natural ingredients of Sanjin Tablets. These compounds presented essentially no antibacteria activity, indicating that they affect UPEC by means other than directly killing the cells. Further molecular modeling analysis predicted molecular targets for these compounds and mapped the residues potentially involved in compound-target interactions. All the predicted targets turned out to be critical proteins regulating the metabolisms of *E. coli*, suggesting that these compounds may affect metabolic pathways in UPEC and inhibit pathogenesis. These data will benefit future design of drugs with higher efficacy and specificity on targeting pathogenic bacteria.

1. Introduction

The urinary tract infection (UTI) is a most common infectious disease caused by the inflammation of the renal system and characterized by frequent and painful urination. UTI is associated with significant morbidity and mortality, with an annual occurrence of approximately 150 million cases [1]. The lifetime risk for women having UTI is 40%–50%, approximately 10-fold higher than that for men. The most common cause for UTI is uropathogenic *Escherichia coli* (UPEC), which follows a multistep pathogenic scheme to induce UTI, namely, adhesion/colonization of local mucosa, evasion of host defenses, multiplication, and host damage [2]. Extensive studies have been devoted to understanding the molecular mechanisms of UPEC pathogenesis, with the goal to develop efficient therapy targeting UTI.

Traditional Chinese medicine (TCM) has a long history of successful applications on various human diseases. With thousands of years of empirical knowledge, TCM is not static but is rather undergoing rapid transformation to continue successfully in a changing world [3]. With the explosion of biomedical sciences during the past two decades, it is realized that TCM represents an extensive database of chemical compounds that could be utilized for rational drug design [4–6].

Sanjin Tablets developed by Guilin Sanjin Pharmaceutical Co., Ltd., are an all-natural herbal preparation with demonstrated efficacy in treating urinary tract infection [7]. The ingredients for Sanjin Tablets include *Rosa laevigata*, *Melastoma normale* D. Don, *Smilax china*, *Lygodium japonicum*, and *Centella asiatica*. However, it is not known what compounds within these ingredients are effective for preventing UPEC pathogenesis and treating urinary tract infection. In this study, we applied affinity binding to separate components from Sanjin Tablets that bind UPEC soluble proteins and used liquid chromatography-mass spectrometry (LC-MS) to

figure out molecular formulas of these components. The molecular formulas of eight compounds were predicted, and their structures were identified by comparing their MS spectra profiles with that of known Sanjin Tablets compounds using the internal MS spectrum database established by Guilin Sanjin Pharmaceutical Co., Ltd. Functional analysis showed that these compounds presented substantially no bacteria-killing activity, and *in silico* screening predicted molecular targets of these compounds to be important *E. coli* proteins involved in metabolism, suggesting that these compounds could potentially block metabolic pathways in UPEC and inhibit pathogenesis. Identification of these potential bioactive compounds in Sanjin Tablets will benefit future design of drugs with higher efficacy and specificity to the UTI-causing uropathogenic *E. coli*.

2. Materials and Methods

2.1. Preparation of Soluble Protein Solution from UPEC. The pathogenic UPEC strain was obtained from Department of Microbiology, School of Preclinical Medicine, Guangxi Medical University (Guangxi, China). The UPEC cells were cultured in Luria-Bertani (LB) broth medium (10 g peptone, 5 g yeast extract, and 5 g sodium chloride per liter) at 37°C until the cell density reached 3-4 $\times 10^8$/mL and were harvested by centrifugation at 5,200 ×g for 15 min at 4°C. The cell pellet was resuspended in PBS buffer containing PMSF and DNase I, and the cells were lysed by sonication on ice. The cell lysate was centrifuged at 34,000 ×g for 20 min at 4°C to clear off insoluble proteins, most lipids, and large carbohydrates, and the supernatant containing the soluble proteins was dialyzed against PBS for 24 h at 4°C with a 3.5 kDa molecular weight cutoff (MWCO) membrane (Millipore, Billerica, MA, USA), followed by a second-round dialysis in 3.5 kDa MWCO membrane against PBS with 0.5 M NaCl for another 24 h at 4°C to completely remove the free small endogenous molecules (ribonucleotides, small carbohydrates, etc.). The resulting UPEC soluble protein solution was then collected from inside the dialysis membrane.

2.2. Affinity Binding of Sanjin Tablets Components to Soluble E. coli Proteins. Sanjin Tablets (Guilin Sanjin Pharmaceutical Co., Ltd., Guilin, China) were dissolved in Milli-Q water at a final concentration of 0.06 g/mL, mixed with the UPEC soluble protein solution as prepared above at 1 : 5 (v/v) ratio, and dialyzed in 3.5 kDa MWCO membrane against PBS for 24 h at 4°C to remove unbound small molecules. As control, Milli-Q water alone (without Sanjin Tablets) was added to the UPEC soluble protein solution at 1 : 5 (v/v) ratio and dialyzed the same way.

2.3. Separation of Compounds Bound to Soluble UPEC Proteins. Compounds bound to the UPEC proteins were eluted by adjusting the buffer pH to 1.0 with HCl. The small-molecule compounds in the eluate were separated from other macromolecules by passing through a 3.5 kDa MWCO dialysis membrane at 4°C. Then the buffer pH of the flow-through was adjusted to pH 7.0 with Tris Base powder. The final volume of the flow-through was about 120% of the volume of the original Sanjin Tablets-UPEC soluble protein solution mixture.

2.4. Identification of Compounds Bound to UPEC Soluble Proteins. The flow-through containing Sanjin Tablets compounds capable of binding to UPEC soluble proteins was analyzed by HPLC C18 column (Agilent Zorbax SB-C18, 150 mm × 4.6 mm, 5 μm, CA, USA). The HPLC settings were as follows: mobile phase: 100% acetonitrile; flow rate: 1 mL/min; absorbance: 300 nm; column temperature: 30°C; injection volume: 10 μL. The fractions with UV signals were pooled and the acetonitrile was removed by rotary evaporation, and the dried material was dissolved in methanol to 0.5 mL for further analysis by liquid chromatography-mass spectrometry (LC-MS, ACQUITY UPLC & Q-TOF MS Premier, Waters, USA). The LC-MS settings were as follows: mobile phase A: acetonitrile (10%–90%); mobile phase B: Milli-Q water (90%–10%, with 0.1% formic acid); time: 45 min; flow rate: 0.2 mL/min; sample loading volume: 10 μL; absorbance: 283 nm; ESI mode: positive ionization full-scan mode; mass-to-charge (m/z) range: 100–1,700; nebulizer and drying gas: liquid nitrogen; collision gas: high purity nitrogen; drying gas flowing rate: 8.0 L/min; drying gas temperature: 350°C; nebulizer: 30 psig; capillary voltage: 3,500 V; fragmentor voltage: 175 V; collision energy: 40/70 V. The data were analyzed using the Masslynx 4.1 software [8].

2.5. Determination of Minimum Inhibitory Concentration (MIC). The MIC of each compound with binding potential for UPEC was determined using a microtiter dilution plate method as described previously [9]. Briefly, the compounds were diluted in LB medium to 20 mg/mL, and 2-fold dilutions were prepared with LB medium in the wells of a microtiter plate. *E. coli* was incubated in LB medium at 37°C and 200–250 rpm shaking until OD_{600} reached 0.6, and the culture was inoculated at 1 : 200 (v/v) into the LB media with diluted compounds on the microtiter plate. The plate was incubated at 37°C for 16 hours and the growth of *E. coli* was determined visually. All MIC determinations were repeated two times in independent experiments. The *E. coli* strains used were the nonpathogenic DH5α (New England Biolabs, Ipswich, MA) and the pathogenic UPEC. The broad-spectrum antibiotic ampicillin (iScience, LCP Biomed Co., Lianyungang, China) was used as the positive control.

2.6. Molecular Modeling Studies. The PharmMapper Server was used to predict targets for each compound based on over 7,000 receptor-based pharmacophore models that cover information on 1,627 drug targets [10]. The *E. coli* protein with the highest target score was selected for docking. Molecular docking was performed using MOE 2014.09 (Chemical Computing Group Inc., Montreal, Canada) with the standard induced-fit protocol provided in the software.

3. Results

3.1. Separation of Compounds from Sanjin Tablets That Bind to UPEC Soluble Proteins. It has been established that Sanjin Tablets exhibit inhibitory role on bacterial infections,

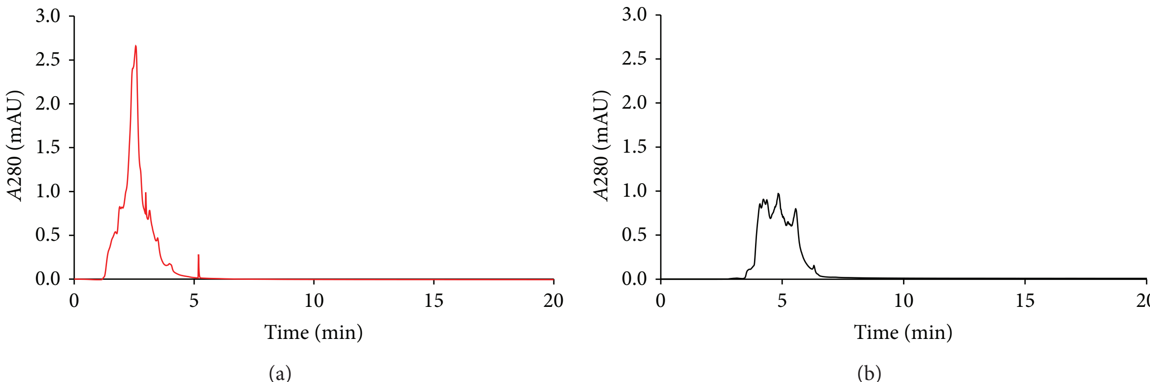

FIGURE 1: HPLC profiles of the *E. coli* protein-bound compounds from (a) Sanjin Tablets and (b) water control. HPLC column: C18. Mobile phase: 100% acetonitrile. Flow rate: 1 mL/min. UV wavelength: 300 nm.

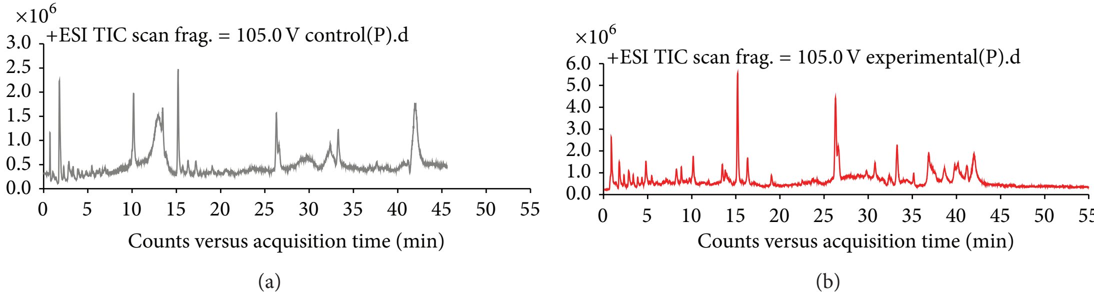

FIGURE 2: Total ion chromatograms of LC-MS analysis of Sanjin Tablet eluate. ESI ion mode: positive. Red line: Sanjin Tablet eluate. Gray line: water only control eluate.

especially in the urinary tract [7]. The pathogenic UPEC strain is a significant target of Sanjin Tablets. To identify the bioactive compounds targeting UPEC from Sanjin Tablets, we carried out an affinity-based separation of compounds from Sanjin Tablets that bind to UPEC soluble proteins. For this purpose, the UPEC cells were lysed by sonication with the presence of DNase I. During the cell lysis procedure, nucleic acid should be digested, cell membrane should be disrupted, and the carbohydrates composing the cell wall should be destructed. The insoluble components including insoluble proteins, lipids, large carbohydrates, and other cell debris were removed by high-speed centrifugation, and the water-soluble components were dialyzed through 3.5 kDa MWCO membranes to remove the free small endogenous molecules like ribonucleotides and small carbohydrates. After dialysis, the solution containing mostly the UPEC soluble proteins was incubated with the water solution of Sanjin Tablets to allow affinity binding between components from Sanjin Tablets to UPEC soluble proteins to occur. The protein-bound compounds were then eluted by adjusting the pH to 1.0. The compounds in the eluate were analyzed by HPLC. Small endogenous molecules that bound to UPEC soluble proteins were also eluted, but their peaks would appear in both the HPLC profiles of the eluate of Sanjin Tablets-UPEC mixture and that of the water-UPEC mixture. Only the difference of the two HPLC profiles would reflect compounds from Sanjin Tablets that bound to UPEC soluble proteins. Indeed, by comparing the HPLC profile of the eluate from Sanjin Tablets-UPEC mixture (Figure 1(a)) with that from water-UPEC mixture (Figure 1(b)), we identified significant difference, suggesting that some components from Sanjin Tablets bound to UPEC soluble proteins, and they were separated successfully.

3.2. Prediction of the Molecular Formulas of Sanjin Tablets Compounds That Bound to UPEC Soluble Proteins. To characterize the compounds from Sanjin Tablets that bound to UPEC soluble proteins, we applied LC-MS technology. At the electrospray ionization (ESI) positive ion mode, the total ion chromatogram of the Sanjin Tablets eluate had notably different areas as compared to those of the control (Figure 2). The MS spectra profiles obtained from LC-MS were screened for matches within the internal MS spectrum database established by Guilin Sanjin Pharmaceutical Co., Ltd., which contained most compounds of Sanjin Tablets. The screening found eight matching MS spectra profiles, which were from known Sanjin Tablets compounds. The mass-to-charge (m/z) ratios of these eight compounds were 287.1, 343.1, 595.2, 611.1, 885.5, 975.5, 271.1, and 465.1, respectively. To confirm that these eight compounds were presented only in the eluate ion chromatogram from Sanjin Tablets but not in the control, these m/z values were used to scan the full retention time of LC-MS of both the Sanjin Tablets eluate and the control (Figure 3). The retention times of these m/z

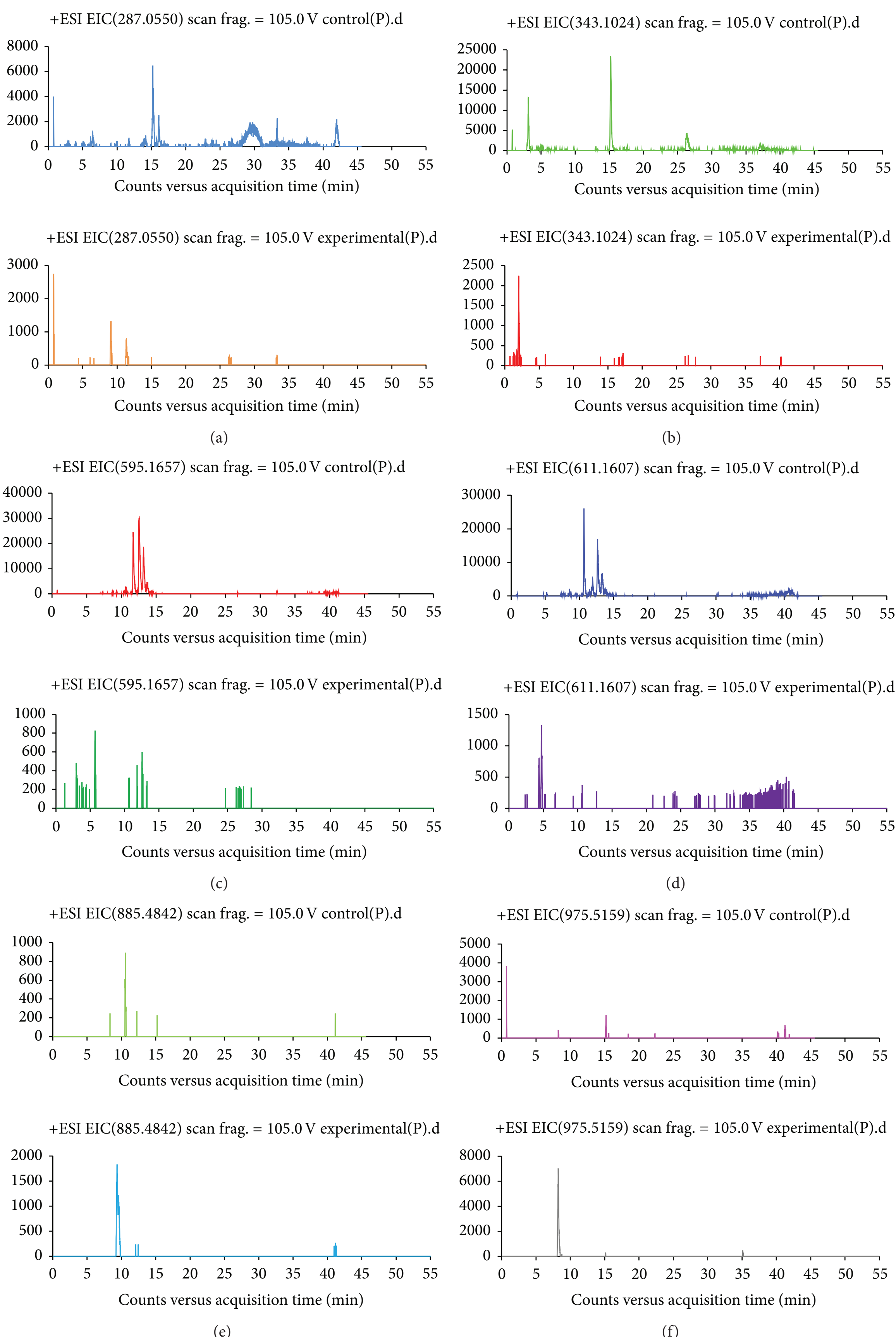

Figure 3: Continued.

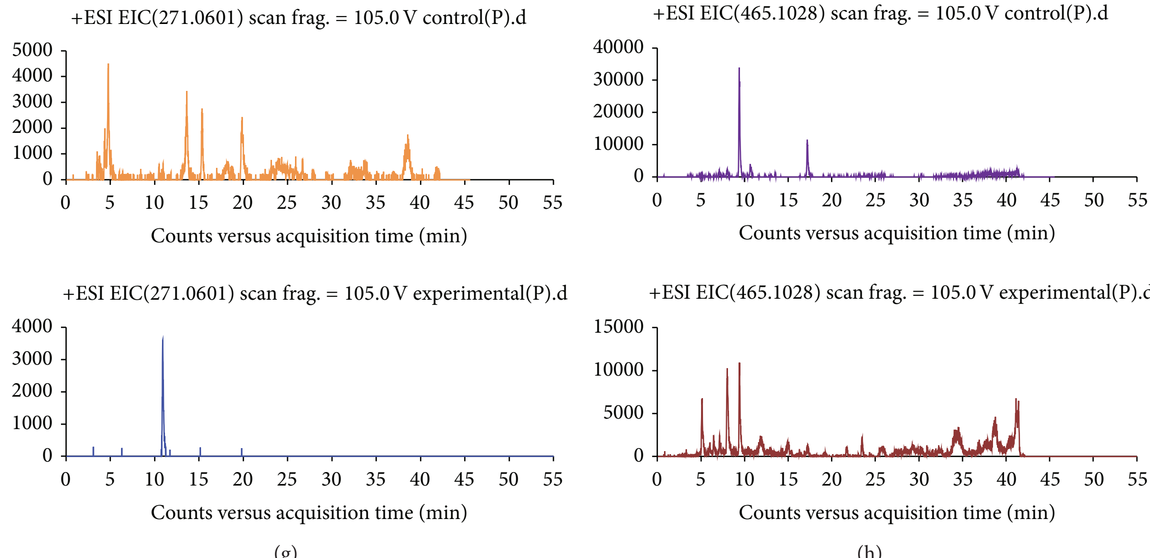

Figure 3: The detailed LC-MS signals of peaks at specific mass-to-charge (m/z) ratios 287.1 (a), 343.1 (b), 595.2 (c), 611.1 (d), 885.5 (e), 975.5 (f), 271.1 (g), and 465.1 (h). Of each panel, signal of the Sanjin Tablets compound was presented at the lower position, and the control signal was presented at the upper position.

signals in the eluate ion chromatogram from Sanjin Tablets were 11.8 min, 2.1 min, 3.1 min, 4.5 min, 9.4 min, 8.6 min, 11.0 min, and 5.2 min, respectively, and there was no signal at these specific retention times in the control, indicating that they are indeed in the Sanjin Tablets eluate only. The mass spectrum of each peak was further analyzed by another round of high-resolution LC-MS using the isolated fractions of these peaks and generated the most possible molecular formula of each compound (Figure 4 and Table 1). The scores in Table 1 represent the confidence of each molecular formula generated.

Table 1: Predicted molecular formulas of Sanjin Tablets compounds.

Compound number	Molecular formula	Score
1	$C_{15}H_{10}O_6$	79.43
2	$C_{15}H_{18}O_9$	82.38
3	$C_{27}H_{30}O_{15}$	74.09
4	$C_{27}H_{30}O_{16}$	72.85
5	$C_{45}H_{72}O_{17}$	88.63
6	$C_{48}H_{78}O_{20}$	95.41
7	$C_{15}H_{10}O_5$	83.24
8	$C_{21}H_{20}O_{12}$	99.65

3.3. Identification of Sanjin Tablets Compounds. The high-resolution MS spectra profiles of the eight compounds as well as their predicted molecular formulas were compared again with the above screening matches of known Sanjin Tablets compounds, and, fortunately, we found that all the molecular formulas and the MS spectra profiles matched well. Thus we identified these eight UPEC protein-binding compounds from Sanjin Tablets, and because the natural sources of these compounds were available in the database of Guilin Sanjin Pharmaceutical Co., Ltd., their original ingredients were figured out (Table 2). Compounds 1 and 5 were from *Smilax china*; Compounds 2, 3, 4, and 8 were from *Lygodium japonicum*; Compound 6 was from *Centella asiatica*; and Compound 7 was from *Rosa laevigata*.

3.4. Measurement of the Antibacteria Activity of the Isolated Compounds. In order to explore the antibacteria activity of the Sanjin Tablet compounds, all eight compounds were purchased from commercial suppliers and tested for their individual antibacteria activities. We used a microtiter dilution plate method and quantitatively determined the minimum inhibitory concentration (MIC) of each compound for *E. coli* strain DH5α. As shown in Table 3, only Compounds 2 and 4 exhibited weak antibacteria activity with MIC of 2.5 mg/mL and 10 mg/mL, respectively. When compared with the MIC of control ampicillin (0.005 mg/mL), the antibacteria activities of Compounds 2 and 4 were 500-fold and 2,000-fold lower, respectively. The other compounds did not exhibit any inhibition on *E. coli* growth, even at the highest concentration used (20 mg/mL). The same MIC values were obtained when we used the pathogenic UPEC strain.

Among these compounds, several were tested for their antibacteria activities in other studies and had been reported. According to these reports, Compound 3 did not show obvious antibacteria activity, while Compound 4 showed minimal activity with an MIC value similar to our result [11, 12]. Compound 8 was reported to have weak antibacteria activity, but the MIC value was not quantitatively determined [12]. Only Compound 1 was recognized to be an antibacterial molecule, but the MIC values from different studies varied significantly [13], suggesting that Compound 1 had strain preference. The artificially engineered DH5α strain and the

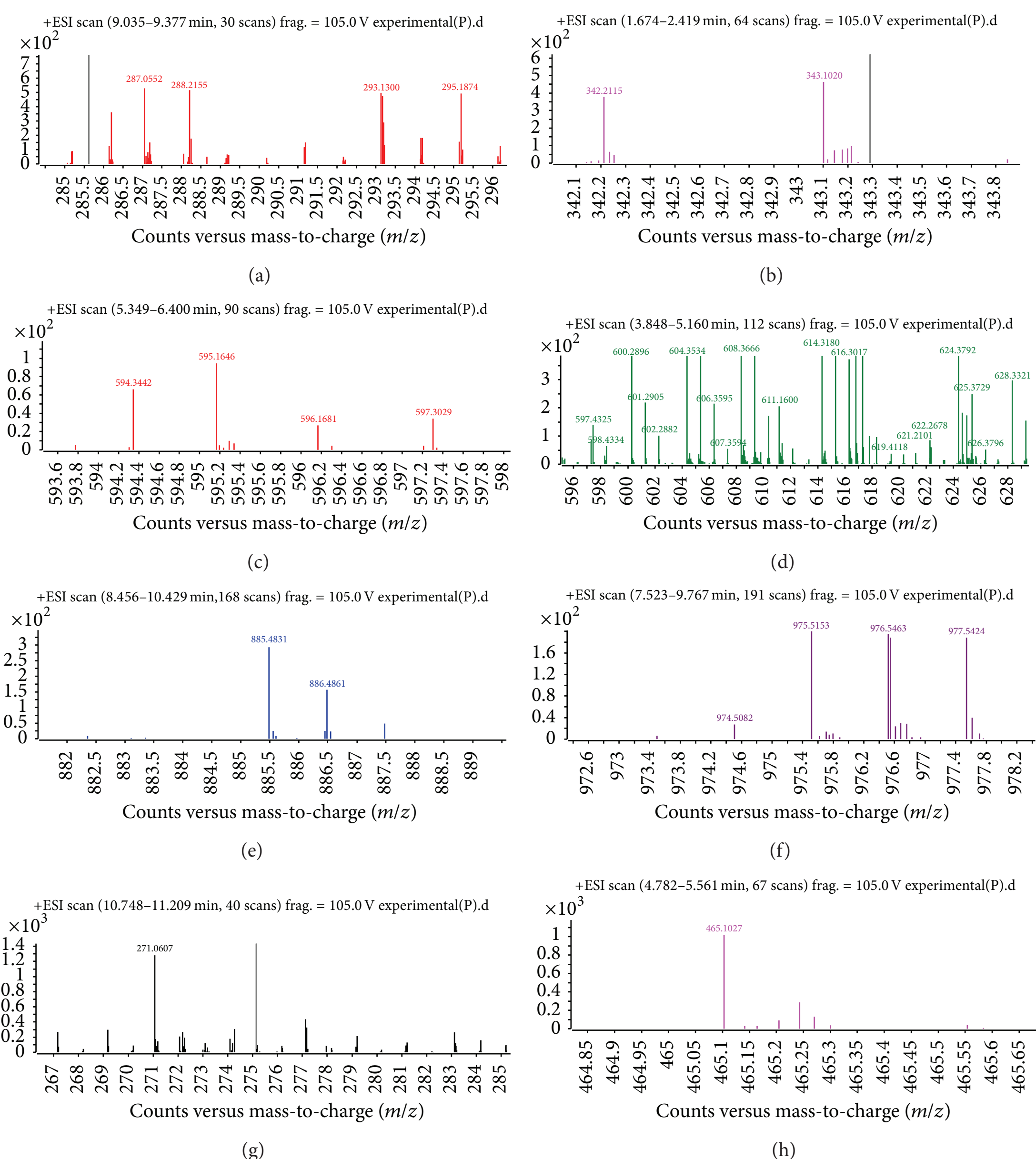

FIGURE 4: High-resolution MS spectra of the LC-MS peaks that only presented in Sanjin Tablets eluate with molecular ion peaks at mass-to-charge (m/z) ratios 287.1 ((a) retention time 11.8 min), 343.1 ((b) retention time 2.1 min), 595.2 ((c) retention time 3.1 min), 611.1 ((d) retention time 4.5 min), 885.5 ((e) retention time 9.4 min), 975.5 ((f) retention time 8.6 min), 271.1 ((g) retention time 11.0 min), and 465.1 ((h) retention time 5.2 min).

antibiotic-resistant UPEC strain used in this study may be insensitive to it.

We further tested the antibacteria activity of the water solution of Sanjin Tablets (62.5 mg/mL) against UPEC, which showed essentially no antibacteria activity as well (data not shown). Our results combined with previous reports suggested that Sanjin Tablets or the UPEC lysate-binding compounds are not directly inhibiting bacterial growth but rather antagonize bacteria through other mechanisms.

3.5. In Silico Target Prediction of Sanjin Tablets Compounds. To further study the mechanisms on how the eight potential bioactive compounds of Sanjin Tablets affect *E. coli*, PharmMapper Server was used to predict potential targets for these eight compounds. The predicted *E. coli* targets with highest scores were all important proteins in *E. coli* metabolic pathways (Table 4). Among them, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase is a member of the cytidylyltransferase family, an essential enzyme in the mevalonate-independent pathway of isoprenoid biosynthesis [14]; maltose-binding periplasmic protein is a periplasmic binding protein responsible for transport of maltooligosaccharides through the periplasmic space, as a part of the ABC transport system [15]; glutamine-fructose-6-phosphate aminotransferase acts as a nucleophile to release and transfer ammonia from glutamine to fructose 6-phosphate

Table 2: Information of Sanjin Tablets compounds.

Compound number	Name	Alternative names	Molecular formula	Molecular weight	Structure
1	Kaempferol	Robigenin, Pelargidenolon, Rhamnolutein, Rhamnolutin, Populnetin, Trifolitin, Kaempferol, and Swartziol	$C_{15}H_{10}O_6$	286.23	
2	6-O-Caffeoyloxy-glucopyranoside	n/a	$C_{15}H_{18}O_9$	342.30	
3	Kaempferol 3-O-rutinoside	Kaempferol 3-O-rhamnosyl-glucoside, Nicotiflorine, and Kaempferol 7-neohesperidoside	$C_{27}H_{30}O_{15}$	594.52	
4	Rutin	Rutoside, Quercetin-3-O-rutinoside, and Sophorin	$C_{27}H_{30}O_{16}$	610.52	
5	Gracillin	n/a	$C_{45}H_{72}O_{17}$	884.48	
6	Madecassoside	Asiaticoside A and Madecassoside redermic	$C_{48}H_{78}O_{20}$	975.13	

Table 2: Continued.

Compound number	Name	Alternative names	Molecular formula	Molecular weight	Structure
7	1,3,8-Trihydroxy-6-methylanthraquinone	Emodin and Rheum emodin	$C_{15}H_{10}O_5$	270.24	
8	Isoquercitrin	Isoquercitroside, Trifoliin, Isotrifolin, Trifoliin A, Isohyperoside, Isotrifoliin, and Quercetin-3-glucoside	$C_{21}H_{20}O_{12}$	464.38	

Table 3: Minimum inhibitory concentrations (MIC) of Sanjin Tablets compounds to *E. coli*.

Compound number	Name	Minimum inhibitory concentration (MIC, mg/mL)
1	Kaempferol	>20
2	6-O-Caffeoyloxy-glucopyranoside	2.5
3	Kaempferol 3-O-rutinoside	>20
4	Rutin	10
5	Gracillin	>20
6	Madecassoside	>20
7	1,3,8-Trihydroxy-6-methylanthraquinone	>20
8	Isoquercitrin	>20
Control	Ampicillin	0.005

through a channel [16]; 4-aminobutyrate aminotransferase is a tetrameric pyridoxal phosphate-dependent enzyme that catalyzes transamination between primary amines and R-keto acids [17]; N-acetyl-L-glutamate kinase catalyzes the phosphorylation of the gamma-COO(-) group of N-acetyl-L-glutamate (NAG) by ATP [18].

3.6. Molecular Modeling of Target-Compound Docking. To map the potential interactions between compounds from Sanjin Tablets and the *E. coli* targets as suggested by PharmMapper analysis. We used MOE 2014.09 software to dock the compounds onto the *E. coli* target protein structures. The induced-fit docking illustrated that these compounds achieved high scores of docking and formed hydrogen bonds with the residues in the binding pockets of target protein (Figure 5 and Table 5). Furthermore, the binding of the compounds all occurred at the critical positions for enzymatic activities of their targets. For example, Compound 3 (Kaempferol 3-O-rutinoside) bound to the enzymatic active site of the glutamine-fructose-6-phosphate aminotransferase and formed hydrogen bonds with residues Ser303, Gln348, Thr352, and Glu488, which could substantially inhibit aminotransferase activity of the target (Figure 5(c)).

4. Discussion

In this study, we identified eight compounds of Sanjin Tablets that bound to the soluble proteins of UPEC. By antibacteria functional assay, we determined that these compounds were not directly killing the target bacteria. Through computer-based target screening and docking site mapping, we predicted that these compounds all work on proteins essentially regulating the metabolism of *E. coli*, suggesting that targeting the metabolism is a key mechanism by which Sanjin Tablets present their actions on UTI.

The effects of Sanjin Tablets in treating UTI are well-established. However, it is unclear through which mechanism(s) that Sanjin Tablets antagonize UPEC infection and treat UTI. Unawareness of bioactive compounds in Sanjin Tablets makes the safety of using Sanjin Tablets for UTI under debate [19]. To further develop Sanjin Tablets into novel UTI-treating drugs with known components and accurately established safety and specificity, it is important to identify bioactive compounds contained in Sanjin Tablets and understand their mechanisms in targeting UPEC. In our preliminary study, we found that when applying the soluble solution of Sanjin Tablets directly onto living *E. coli*, Sanjin Tablets have no killing effect, suggesting that

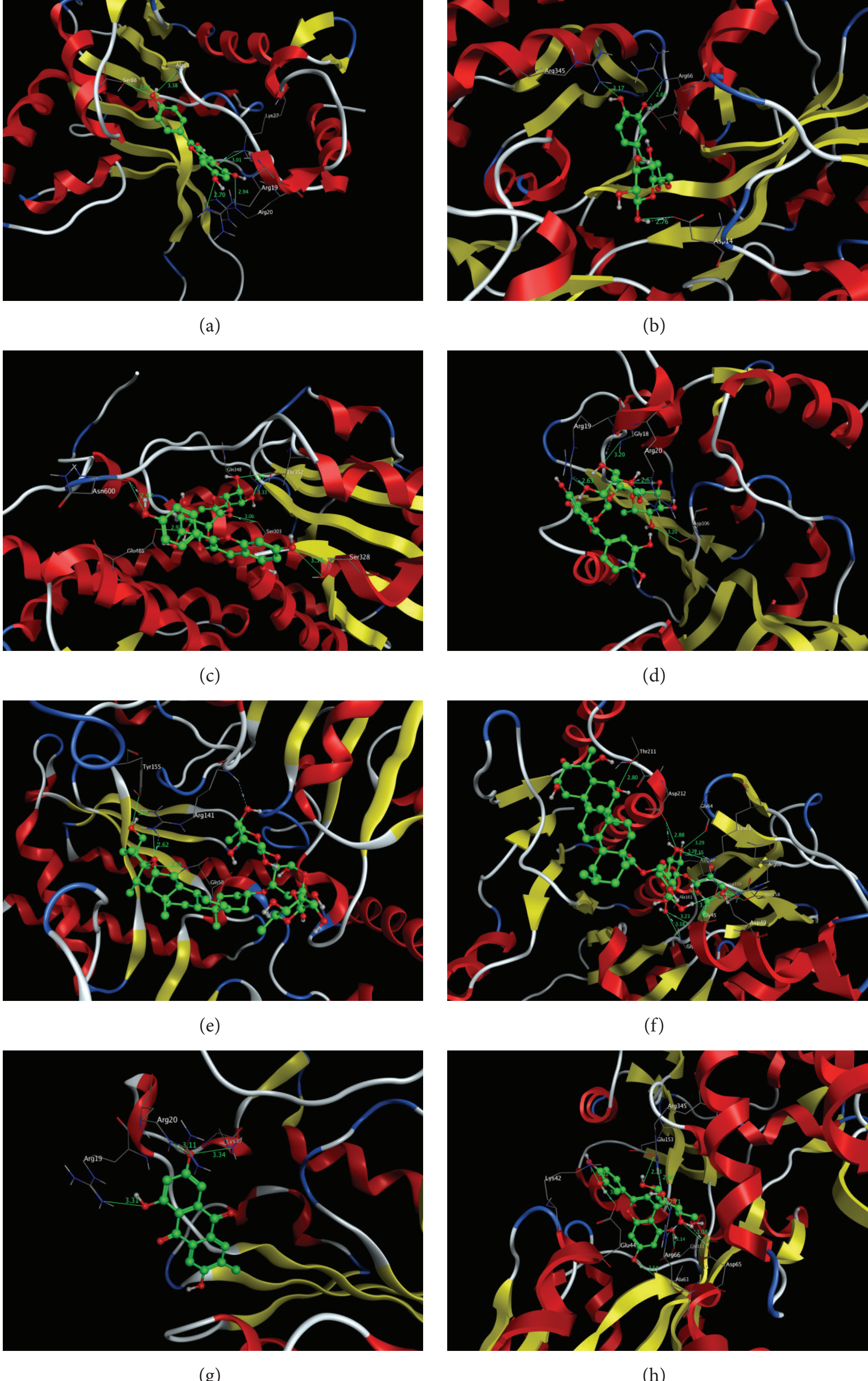

Figure 5: Docking results of Sanjin Tablet compounds into the binding pockets of their targets. (a) Complex of Kaempferol with 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (PDB ID 1I52). (b) Complex of 6-O-caffeoyloxy-glucopyranoside with maltose-binding periplasmic protein (PDB ID 1JVY). (c) Complex of Kaempferol 3-O-rutinoside with glutamine-fructose-6-phosphate aminotransferase (PDB ID 1MOS). (d) Complex of Rutin with 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (PDB ID 1I52). (e) Complex of orbiculatoside B with 4-aminobutyrate aminotransferase (PDB ID 1SZS). (f) Complex of Madecassoside with Acetylglutamate kinase (PDB ID 1OHA). (g) Complex of 1,3,8-trihydroxy-6-methylanthraquinone with 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (PDB ID 1I52). (h) Complex of Isoquercitrin with maltose-binding periplasmic protein (PDB ID 1JVY). Only the pose of binding pocket was shown for each compound-target complex. Color designations: Sanjin Tablet compounds: green; α-helices: red; β-sheets: yellow; turns: blue; loops: white.

Table 4: The highest *E. coli* targets predicted for Sanjin Tablets compounds.

Compound number	Compound name	Highest *E. coli* target	
		Name	PDB ID
1	Kaempferol	2-C-Methyl-D-erythritol 4-phosphate cytidylyltransferase	1I52
2	6-O-Caffeoyloxy-glucopyranoside	Maltose-binding periplasmic protein	1JVY
3	Kaempferol 3-O-rutinoside	Glutamine-fructose-6-phosphate aminotransferase	1MOS
4	Rutin	2-C-Methyl-D-erythritol 4-phosphate cytidylyltransferase	1I52
5	Gracillin	4-Aminobutyrate aminotransferase	1SZS
6	Madecassoside	Acetylglutamate kinase	1OHA
7	1,3,8-Trihydroxy-6-methylanthraquinone	2-C-Methyl-D-erythritol 4-phosphate cytidylyltransferase	1I52
8	Isoquercitrin	Maltose-binding periplasmic protein	1JVY

Table 5: Docking scores and interactions of Sanjin Tablets compounds to the predicted targets.

Compound number	Target PDB ID	Docking score	Number of hydrogen bonds	Residues involved
1	1I52	−5.6899	5	Ala15, Arg19, Arg20, Lys27, and Ser88
2	1JVY	−6.8822	4	Asp14, Asp65, Arg66, and Arg345
3	1MOS	−7.2693	6	Ser303, Ser328, Gln348, Thr352, Glu488, and Asn600
4	1I52	−7.7983	4	Gly18, Arg19, Arg20, and Asp106
5	1SZS	−8.3089	3	Gln50, Arg141, and Tyr155
6	1OHA	−8.6682	12	Gly44, Gly45, Asp49, Lys61, Gly64, Arg66, Asn158, Val159, Asn 160, Ala161, Thr211, and Asp212
7	1I52	−5.4116	3	Arg19, Arg20, and Lys27
8	1JVY	−8.1743	8	Lys42, Glu44, Ala63, Asp65, Arg66, Glu111, Glu153, and Arg345

their antibacterial activity might be mediated through other mechanisms. Considering that, following digestion in the human body, Sanjin Tablets might be disintegrated into small-molecule compounds that are easy to enter the bacteria and function intracellularly, we decided to use the soluble proteins lysed from UPEC as the bait to fish out compounds from Sanjin Tablets that possess the capability of binding to UPEC soluble proteins. By combining affinity binding and LS-MS data, we identified eight compounds that interacted with UPEC soluble protein. Moreover, similar to Sanjin Tablets, these compounds presented minimal or no direct antibacterial activity, corroborating that Sanjin Tablets inhibit UPEC through mechanisms other than directly enhancing apoptosis and reducing proliferation.

Using computer-based target screening analysis, we predicted potential interacting partners for each of the eight compounds. All the potential interacting partners turned out to be critical proteins regulating the metabolisms of *E. coli*. Furthermore, the docking site analysis confirmed that, following the binding of the compounds, essential residues for the enzyme activities of the metabolism proteins are often blocked, thereby suggesting that Sanjin Tablets, through their component compounds, may block metabolic pathways in UPEC and inhibit the pathogenesis.

Among the predicted interacting partners, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase is an essential enzyme in the mevalonate-independent pathway of isoprenoid biosynthesis, also known as the methylerythritol phosphate 1 pathway [20]. In *E. coli*, it catalyzes the conversion of 2-C-methyl-D-erythritol 4-phosphate (MEP) and cytidine triphosphate (CTP) to 4-diphosphocytidyl-2-C-methylerythritol (CDPME). Given its importance in bacterial function, the MEP pathway constitutes an attractive pathway for antibacterial therapy [21]. The 4-aminobutyrate aminotransferase is the *E. coli* isozyme of γ-aminobutyrate aminotransferase (GABA-AT), a tetrameric pyridoxal phosphate-dependent enzyme that catalyzes transamination between primary amines and α-keto acids. GABA-AT is the initial enzyme of GABA-degradation pathway [22], playing critical role in redirecting GABA into TCA cycle [23]. Maltose-binding periplasmic protein (MBP) is a large (370-amino acid residue) bacterial periplasmic protein involved in active transport of and chemotaxis toward maltose. In *E. coli*, upon the initial binding to maltose, MBP undergoes substantial conformational changes to allow maltose to attach to the ATP-binding cassette (ABC) transporters, initiating downstream signaling for either transport or chemotaxis [24]. Glutamine-fructose-6-phosphate aminotransferase (GFAT) is the first and rate-limiting enzyme in the hexosamine biosynthetic pathway, and it controls the flux of glucose through the hexosamine pathway [25]. It catalyzes the conversion of D-fructose-6P into D-glucosamine-6P using L-glutamine as nitrogen donor. In the absence of glucosamine-6P, the deletion of the GFAT gene is lethal in fungi and bacteria, and the inhibition of GFAT enzyme activity could lead to the development of specific antibiotic or antifungal drugs. Targeting the human GFAT enzyme could reduce the flux of intracellular glucose and limit complications associated with

diabetes [26]. N-Acetyl-L-glutamate kinase (NAGK) belongs to the amino acid kinase family and catalyzes the second and the controlling step of arginine biosynthesis [18].

These predicted interacting targets are all in essential metabolic pathways, and complete blocking of their functions may lead to death of the *E. coli* cell. That neither Sanjin Tablets nor their compounds were able to kill the bacteria suggested that binding of the Sanjin Tablets compounds could not completely inhibit the functions of the targets but only give a partial effect to the metabolism. It has been reported that when using Sanjin Tablets to treat UTI, it is difficult for UPEC cells to adhere/colonize onto the local mucosa [27], which is a typical behavior of metabolism-compromised pathogenic bacteria.

Although we have been focusing on using soluble proteins from UPEC as the bait, we should not overlook the possibility and importance of insoluble proteins from UPEC in interacting with Sanjin Tablets components and mediating their antibacterial activities. Given that insoluble proteins often denature and form inclusion body during cell lysis, which prevents optimal binding to compounds from Sanjin Tablets, optimization of the lysis condition for acquiring insoluble proteins in their native conformation is important for us to continue discovering Sanjin Tablets compounds that bind to insoluble UPEC proteins.

The computer-based target prediction and docking site mapping have provided valuable information for mechanistic understanding as well as future drug design [6]. However, it is important to realize that such information needs further corroboration with experimental evidence. It is of special interest to perform in-depth analysis on the individual interactions of the Sanjin Tablets compounds and their predicted targets, which will not only quantitatively provide binding affinities, but also shed light on the molecular mechanisms of how these compounds affect the functions of their targets and how the metabolism of the bacteria is changed.

5. Conclusion

We have identified eight potential bioactive compounds from Sanjin Tablets that may act through antagonizing the metabolism of UPEC and suggested possible mechanisms on how Sanjin Tablets show their efficacy in UTI. Mechanistic study of the interactions between these compounds and their targets shall pave the way for further rational design of drugs that will more potently and specifically target UPEC.

Conflict of Interests

The authors have declared that there is no conflict of interests.

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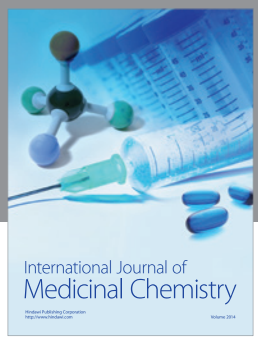

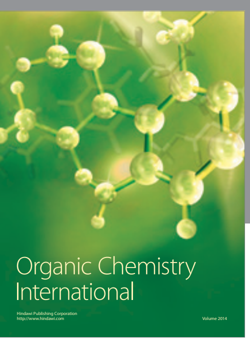

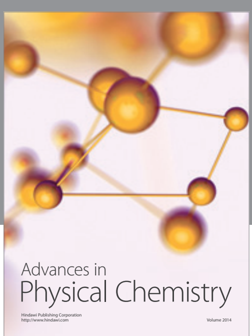

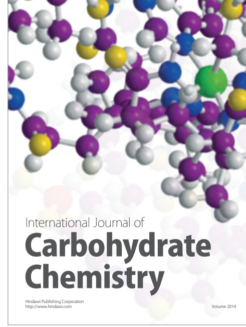

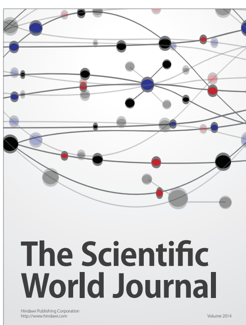

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