Caulis Sinomenii Extracts Activate DA/NE Transporter and Inhibit 5HT Transporter

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***Caulis Sinomenii* (QFT) has analgesic, sedative, and anxiolytic-like actions, and is proven effective for improving drug dependence that is known to be associated with abnormal monoaminergic transmission. We assessed whether QFT would be biologically active in functionally regulating monoamine transporters using CHO cells expressing dopamine transporter (DAT), norepinephrine transporter (NET), or serotonin transporter (SERT) (i.e. D8, N1, or S6 cells, respectively). Here, we showed that its primary extracts, such as QA, QC, QE, QD, and QB (QFT ethanol, chloroform, ethyl acetate, alkaloid-free chloroform, and alkaloid-containing chloroform extract, respectively), and secondary extracts, such as QE-2, −3, −5, −7, QD-1, −2, −3, −4, −5, and QB-1, −2, −3, −4, −5 (fractioned from QE, QD, and QB, respectively), in differing degrees, either increased DA/NE uptake by corresponding D8/N1 cells or decreased 5HT uptake by S6 cells; wherein, QE-2, QD-3, and QE-7 were potent DA/NE uptake activators while both QE-7 and QB-5 were potent 5HT uptake inhibitors. Furthermore, the enhancement of DA/NE uptake was dependent of DAT/NET activity, and the inhibition of 5HT uptake was typical of competition. Thus, QFT extracts, especially QE-2 and QE-7 (both with stronger potencies), are novel monoamine transporter modulators functioning as DAT/NET activators and/or SERT inhibitors, and would likely improve neuropsychological disorders through regulating monoamine transporters.** Exp Biol Med 234:976–985, 2009

Key words: *Caulis Sinomenii*; dopamine; norepinephrine; serotonin; reuptake modulator; transporter

Introduction

Caulis Sinomenii (orientvine stem) is the dried stem of *Sinomenium acutum* (THUNB.) REHD. *et* WILS. or *S. acutum* (THUNB.) REHD. *et* WILS. var. *cinereum* REHD. *et* WILS. (Fam. Menispermaceae) as recorded in the *Chinese Pharmacopoeia* (1). It has been utilized empirically to treat several diseases symptomized by joint pain and swelling in Chinese medicine for over hundreds of years. Pharmacological investigations done by others have demonstrated the anti-inflammatory (2) and anti-rheumatic (3, 4) effects as well as immunosuppressive (5) actions of *Caulis Sinomenii* or its isolates. The folklore herb with oral, chronic dosing is commonly applied for treating rheumatoid arthritis (6) in modern Chinese medicine, and more recently it has been proven effective in preventing the clinical signs of multiple sclerosis (7) and decreasing the degree of preference to morphine (8). Drug dependence is known to be attributable to an abnormal brain-rewarding system that is associated with monoamine dysfunction (9, 10).

Monoamine transmitters, DA, 5HT, and NE, are essential for regulating cognition, affection, and emotion as well as for protecting against neuronal injury (11). Membrane monoamine transporters, i.e. dopamine transporter (DAT), norepinephrine transporter (NET), and serotonin transporter (SERT), belonging to Na^+/Cl^--dependent transporters, act by reuptake of respective extracellular monoamine into presynaptic neurons and thereby play crucial roles in limiting monoaminergic activity in the CNS (12, 13). Maladjustments of monoamine transporters are known to contribute to the imbalance of monoaminergic transmission and thereby triggering the pathologic process of several neuropsychological disorders. According to aforementioned facts, we thus hypothesized that QFT would have bioactivity on monoamine transporters.

In this study, we screened the herb source using a transgenic Chinese hamster ovary (Tr-CHO) cell-line system and discovered that QFT extracts indeed had regulatory actions on monoamine transporters.

This research was supported by a grant from the Shanghai government (05DZ19339) and Chinese Academy of Science.

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Received March 2, 2009.
Accepted April 21, 2009.

DOI: 10.3181/0903-RM-92
1535-3702/09/2348-0976$15.00

Materials and Methods

Preparation of Extracts. *Caulis Sinomenii*, namely Qing Feng Teng (QFT) in Chinese, was bought from Hubei Province, China, and was identified and authenticated as dried stem of *Sinomenium acutum* (THUNB.) REHD. by an expert herbalist at the Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The voucher specimen (no. 20070608) was deposited at the Herbarium of CellStar BioTechnologic Co., Ltd. (Shanghai, China). The specimen was washed, crushed, and homogenized before extraction. Quality control of QFT was conducted by HPLC, which showed that QFT (extracted by ethanol) contains 0.85% Sinomenine, a major QFT active chemical constituent that is commonly used as a chemical indicator for quality evaluation of the plant material (14). The HPLC system for QFT quality control was Agilent1100 series that was equipped with a photodiode array detector (PDAD). An Alltima C18 analytical column coupled with a C18 guard column (300 mm × 3.9 mm, i.d., 10 μm) was used at room temperature of 20°C. The mobile phase was a mixture of acetonitrile, water, and ethylene diamine (25:74.8:0.2) at a flow rate of 1.0 ml/min. The detection wavelength was set at 262 nm. The injection volume was 5 ml. Authentic sinomenine, purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), was dissolved in 70% ethanol.

The specimen (no. 20070608) was diacolated with 95% industrial ethanol, and the resultant was condensed by decompression and an ethanol extract (QA) (yield rate 10.6%) was obtained. Afterwards, the QA was suspended in water, alkalized to pH 10 with ammonia, and then extracted with chloroform, yielding a water layer and a chloroform extract (QC). The former one was further extracted with ethyl acetate and QFT ethyl acetate extract (QE) was obtained thereafter. The last one (QC) was extracted with dilute HCl solution (pH = 3), giving an alkaloid-free chloroform extract (QD) and an acid solution, and the acid solution was re-alkalized to pH 10 with ammonia and further extracted with chloroform, then yielding a chloroform extract rich in alkaloids (QB). QE, QD, and QB were separately fractioned by using silica gel column chromatography [200~300 mesh silica gel (Φ60 × 400 mm); chloroform: methanol = 1:0→10:1, as eluent; 10 ml/tube/8 min as elution speed], yielding four QE fractions (QE-2, –3, –5, and –7), five QD fractions (QD-1, –2, –3, –4, and –5), and five QB fractions (QB-1, –2, –3, –4, and –5). The extraction procedure is summarized in Figure 1.

Cell Culture and Transfection. CHO cells stably expressing the rat DAT, rat SERT, human NET, and mice γ-aminobutyric acid (GABA) transporter (GAT-1), designated as D8, N1, S6, or G1 cells, respectively, were established previously (15, 16). These transgenic CHO cells were used for the current pharmacological experimentation. The screen systems show that 18–20-fold enhancement of neurotransmitter uptake relative to wild-type CHO cells, and saturating concentration of GBR12,935 (GBR; a selective reuptake inhibitor of DAT), desipramine (inhibitor of NET), fluoxetine (inhibitor of SERT), and tiagabine (inhibitor of GAT-1) significantly inhibited the enhanced uptake, confirming the sensitivity and validity of the screen models (17, 18). The cells were grown in RMPI 1640 medium containing 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), *l*-glutamine (2 mM), and G418 (250 μg/ml) on experimentation.

[^{3}H] Labeled Transmitter Uptake Assay. [^{3}H] labeled-DA (10.6 Ci/mmol), NE (38 Ci/mmol), 5HT (104 Ci/mmol), or GABA (88 Ci/mmol) (GE Healthcare UK Limited, Buckinghamshire) uptake was measured by using D8, N1, S6, or G1 cells, respectively, and the performance was carried out according to Standard Operation Procedure (SOP) of Cell Star Bio-Technologies Co., Limited, which was previously established based on the methods described in references (15, 16). For evaluation of the effects of QFT extracts on transporters, different concentrations of drug solutions (diluted with distilled water containing 1% dimethyl sulfoxide in final concentration at pH value of 7.2) together with corresponding [^{3}H] labeled ligand were added at the beginning of the uptake assay.

Mechanism of the enhancing action of QFT extracts on DA/NE uptake was explored using uptake assay. The uptake of [^{3}H]-labeled DA/NE was assayed on the respective D8/N1cells, in which QE-2 together with DAT inhibitor GBR12,935 or QD-3 together with NET inhibitor desipramine were concomitantly added into respective plate wells.

The kinetic properties of 5HT uptake inhibition were evaluated on the S6 platform. The competitive analysis was conducted by using the uptake system starting with addition of a series of concentrations of 5HT (0.02 to 16 μM; a mixture of labeled and unlabeled 5HT), and, at each concentration point of 5HT, QE-7 (10 and 30 μg/ml) or QB-5 (15 and 45 μg/ml) was added. Nonspecific [^{3}H]5HT uptake was determined in the presence of 10 μM fluoxetine in parallel plate wells. Incubation continued for 10 min and was terminated by the addition of ice-cold PBS, and DPM values were detected by a liquid scintillation counting analyzer. Kinetic parameters (Vmax and Km) for [^{3}H]5HT uptake were determined using Origin (edition 7.5) software (Northampton, MA, USA). Nonlinear fitting equation: $y = P1 \times x/(P2 + x)$, x, y, $P1$, and $P2$ representing ligand concentration, uptake value, Vmax, and Km, respectively.

Data Analysis. Data were analyzed using SPSS software v.13.0 (Chicago, IL, USA). Values are expressed as mean ± SEM. Analysis of variance (ANOVA) followed by least significant difference (LSD) *post hoc* tests were used to examine differences between groups. $P < 0.05$ was considered statistically significant.

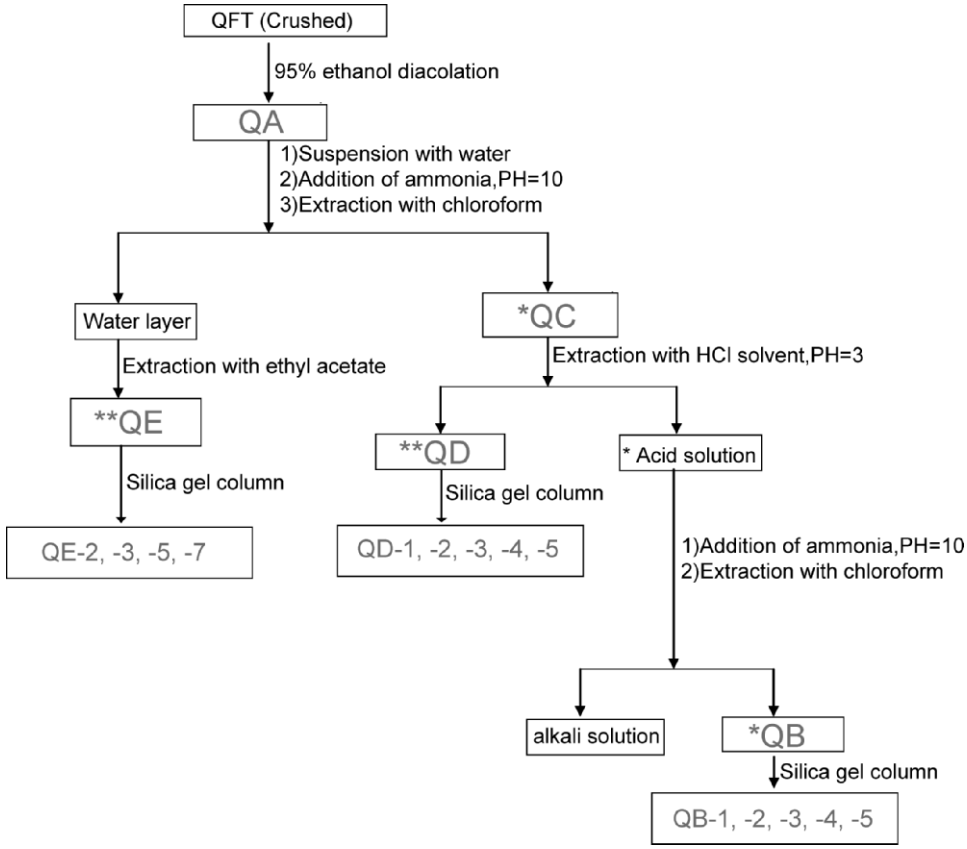

Figure 1. Schematic diagram of QFT fractionation. QA, QFT ethanol extract; QC, chloroform extract; QE, ethyl acetate extract; QD, alkaloid-free chloroform extract; QB, alkaloid-containing chloroform extract. * or **, representing a fraction rich in or devoid of alkaloids, respectively. Each fraction was controlled by thin-layer chromatography (TLC).

Results

QFT Extracts Regulated Monoamine Transport in Tr-CHO. The potential effects of QFT extracts on neurotransmitter transporters were explored by the measurement of isotope-labeled transmitter uptake in corresponding Tr-CHO cells, i.e. D8 cells, S6 cells, G1 cells, and N1 cells (respectively for isotope-labeled DA, 5HT, GABA, and NE uptake), which are demonstrated by corresponding transporter blocker to be suitable for each transporter's assay (Fig. 2A). QA, QC, QE, QD, and QB, at concentration of 100 μg/ml, markedly increased DA uptake by D8 cells (Fig. 2B) or NE uptake by N1 cells (Fig. 2C), with the exception of QB having little action on N1 cells; wherein the DA uptake enhancement by QE and QD, as well as NE uptake enhancement by QD, tended to be more potent (respectively with 4.8-, 5.1-, and 3.1-fold enhancement) (Fig. 2B, 2C). By contrast, QE and QB produced a significant inhibitory effect on 5HT uptake by S6 cells, and the ability of QE to inhibit the 5HT transport seemed to be more potent (Fig. 2D). At concentration of 100 μg/ml, QA, QC, QE, QD, and QB had no action on GABA uptake in G1 cells (Fig. 2E). Thus, QFT extracts may function by selectively regulating monoamine transports in Tr-CHO cells.

To corroborate the finding based on the aforementioned preliminary assessment and to screen a possible fraction with more potency than preliminary extract, QE, QD, or QB was adopted as candidate for further fractionation and activity detection. After silica gel column chromatography, QE, QD, or QB was fractioned into QE-2, –3, –5, and –7 (–1, –4, or –6 was combined with its analogical fraction) (positions shown in TLC Fig. 3A), QD-1, –2, –3, –4, and –5, and QB-1, –2, –3, –4, and –5 (Fig. 1). Uptake assay showed that QE-2, –3, –5, and –7, each at concentration of 100 μg/ml, markedly enhanced DA uptake despite in differential degrees, wherein the potency of QE-2 was strongest which produced a 10-fold enhancement of DA uptake (Fig. 3B); that QE-2 also enhanced NE uptake in N1 cells, but only with a 2.5-fold enhancement (Fig. 3C); and that QE-2, –3, –5, and –7 also produced different degrees of inhibition of 5HT uptake in S6 cells, wherein the potencies of QE-5 and –7 are stronger (Fig. 3D). Compared to QE fractions, QD fractions (QD-1, –2, –3, –4, and –5) produced lower potencies for enhancing DA/NE uptake (Fig. 4A and B) as well as inhibiting 5HT uptake (Fig. 4C), wherein the candidates that possess the most potencies are: QD-3 (with 4- or 2.6-fold enhancement of DA/NE uptake) and QD-1 (with 50% inhibition of 5HT uptake). In cell groups with QB fractions, there are relatively even weaker bioactivities that were only shown in cell groups with QB-1

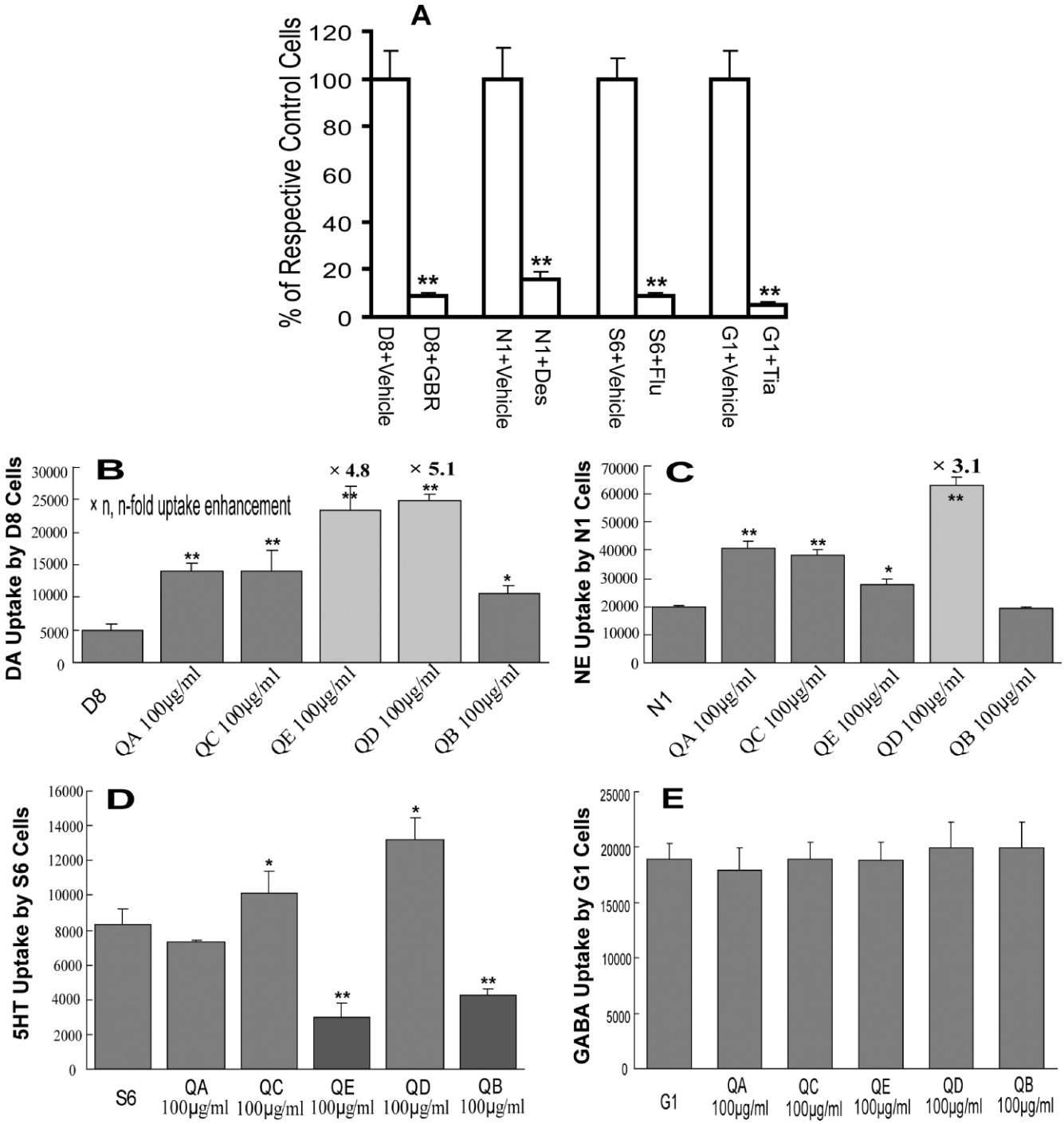

Figure 2. Modulating effects of Qing Feng Teng (QFT) extracts on [^{3}H] labeled transmitter uptake in the Tr-CHO cells. GBR12,935 (GBR, 100 nM), desipramine (Des, 1 μM), fluoxetine (Flu, 10 μM), and tiagabine (Tia, 10 μM), representing selective inhibitors of DAT, NET, SERT, and GAT-1, respectively, markedly inhibited DA/NE/5HT/GABA uptake into corresponding transporter-transgenic CHO cells, demonstrating the feasibility of D8, N1, S6, and G1 cells for evaluating the targeting effects of a drug on respective DAT, NET, SERT, and GAT-1 (A). QFT ethanol extract (QA), chloroform extract (QC), ethyl acetate extract (QE), chloroform extract devoid of alkaloids (QD), and chloroform extract rich in alkaloids (QB), at concentration of 100 μg/ml, markedly increased DA uptake by D8 cells (B) and NE uptake by N1 cells (C), and elevated/inhibited 5HT uptake by S6 cells (D); and all QFT extracts did not affect GABA uptake by G1 cells (E). Y axis is expressed as DPM value, wherein the DPM value for CHO cells is 678.33, 896.56, 956.44, and 1131.66 for the D8, N1, S6, or G1 cell reaction system, respectively. × *n*, *n*-fold uptake enhancement compared to Tr-CHO cells, same below. * and **, $P < 0.05$ and 0.01, respectively, compared to each control cell group. Values are expressed as mean ± SEM of triplicate cell samples.

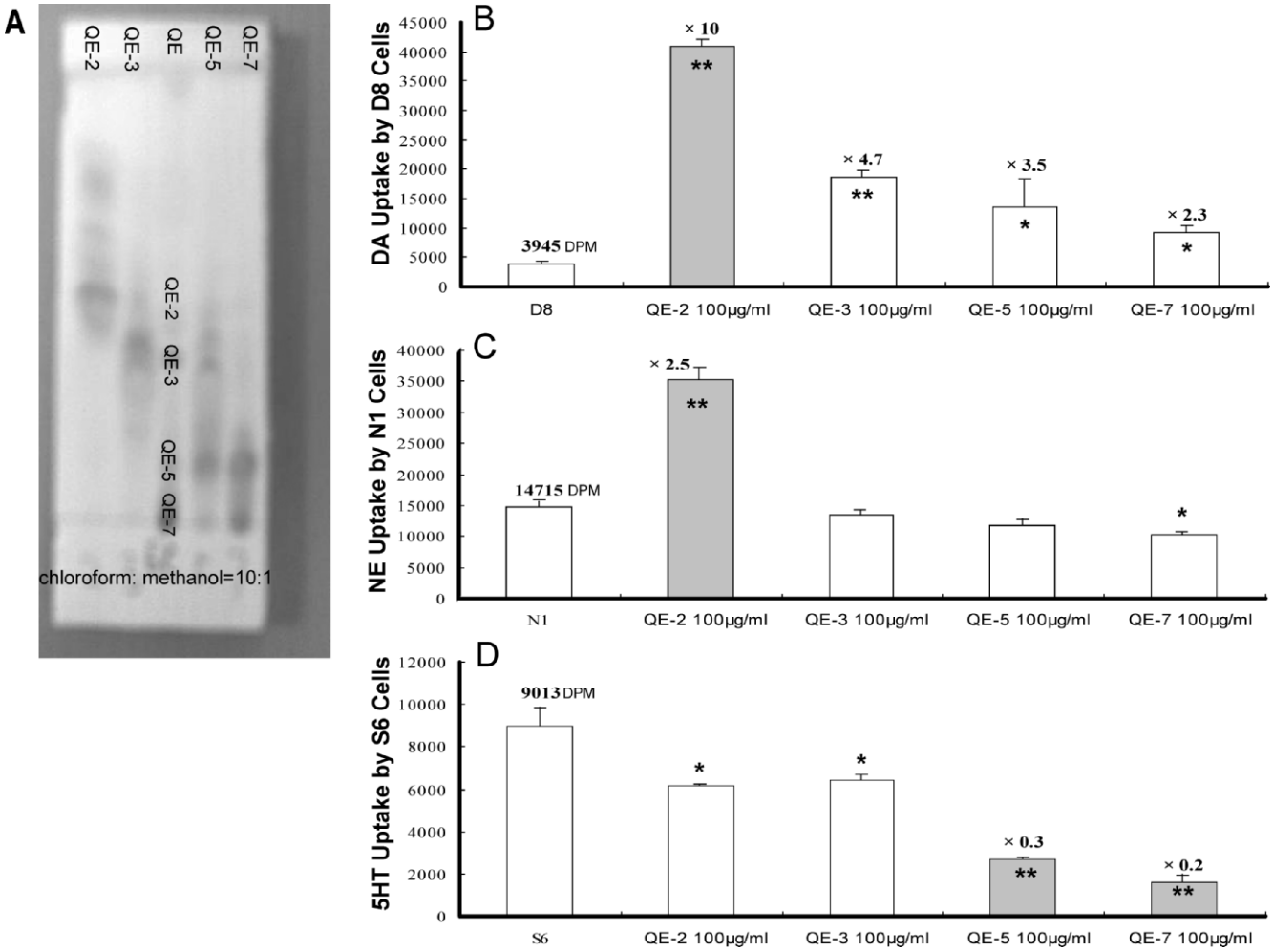

Figure 3. Effects of silica-gel fractions of QFT ethyl acetate extract (QE) on [^{3}H] labeled transmitter uptake in the Tr-CHO cells. TLC shows the positions of QE and fraction QE-2, –3, –5, and –7 (A). Activity detection displays that, at concentration of 100 μg/ml, the fractions QE-2, –3, –5, and –7 strikingly increased DA uptake by D8 cells (B), QE-2 enhanced NE uptake by N1 cells (C), and QE-2, –3, –5, and –7 inhibited 5HT uptake by S6 cells (D). Y axis is expressed as DPM value, which is expressed as mean ± SEM of triplicate cell samples. * and **, $P < 0.05$ and 0.01, respectively, compared to corresponding control cell group.

(mildly enhancing DA/NE uptake and inhibiting 5HT uptake) and QB-5 (moderately inhibiting monoamine uptake) (Fig. 5A–C).

For further corroboration of the uptake-modulating effects and for comparison of bioactivities of differential QFT extracts, the candidate fraction QE-2, QE-7, QD-3, or QB-5, in a broader concentration range (0.02 to 100 μg/ml), was individually added to the screen, and semilogarithmic concentration-effect curve then was obtained. As shown in Figure 6A and C, QE-2 and QD-3 produced concentration-dependent enhancing actions on DA/NE uptake, both with stronger potencies and higher efficacies for DA uptake than for NE uptake. Unlike QE-2 and QD-3, QE-7 showed a mild enhancing action on DA uptake but a stronger inhibitory action on 5HT uptake (Fig. 6B), and QB-5 displayed different levels of potencies for inhibiting DA, NE, and 5HT uptake with more selectivity for 5HT than for DA/NE uptake (Fig. 6D). Besides the enhancing actions in D8/N1 cells, QD-3 also showed a weaker enhancing effect on 5HT uptake in S6 cells when at concentrations up to higher levels (125 to 500 μg/ml) (Fig. 6C). The rank order of potency or efficacy for enhancing DA/NE uptake is: QE-2 > QD-3 > QE-7, and that for inhibiting 5HT uptake is: QE-7 > QB-5.

QFT Extract-Induced Enhancement of Monoamine Uptake Was Inhibited by Transporter Inhibitors. To explore whether the QFT extract-induced enhancement of DA- and NE-uptake could be transporter-dependent, we projected a protocol, in which a selective DAT inhibitor GBR12,935 or NET inhibitor desipramine was used to co-incubate with QE-2 or QD-3. When GBR12,935 (10, 30, and 100 nM) or desipramine (30, 120, and 500 nM) was added to respective Tr-CHO cell line, the enhanced DA/NE uptake produced by QE-2 or QD-3 was markedly and concentration-dependently suppressed (seen in Fig. 7A and B, respectively).

Pharmacokinetic Mechanism of 5HT Uptake Inhibition by QE-7 and QB-5. Saturation analysis in S6-cell-uptake system showed that increasing concentra-

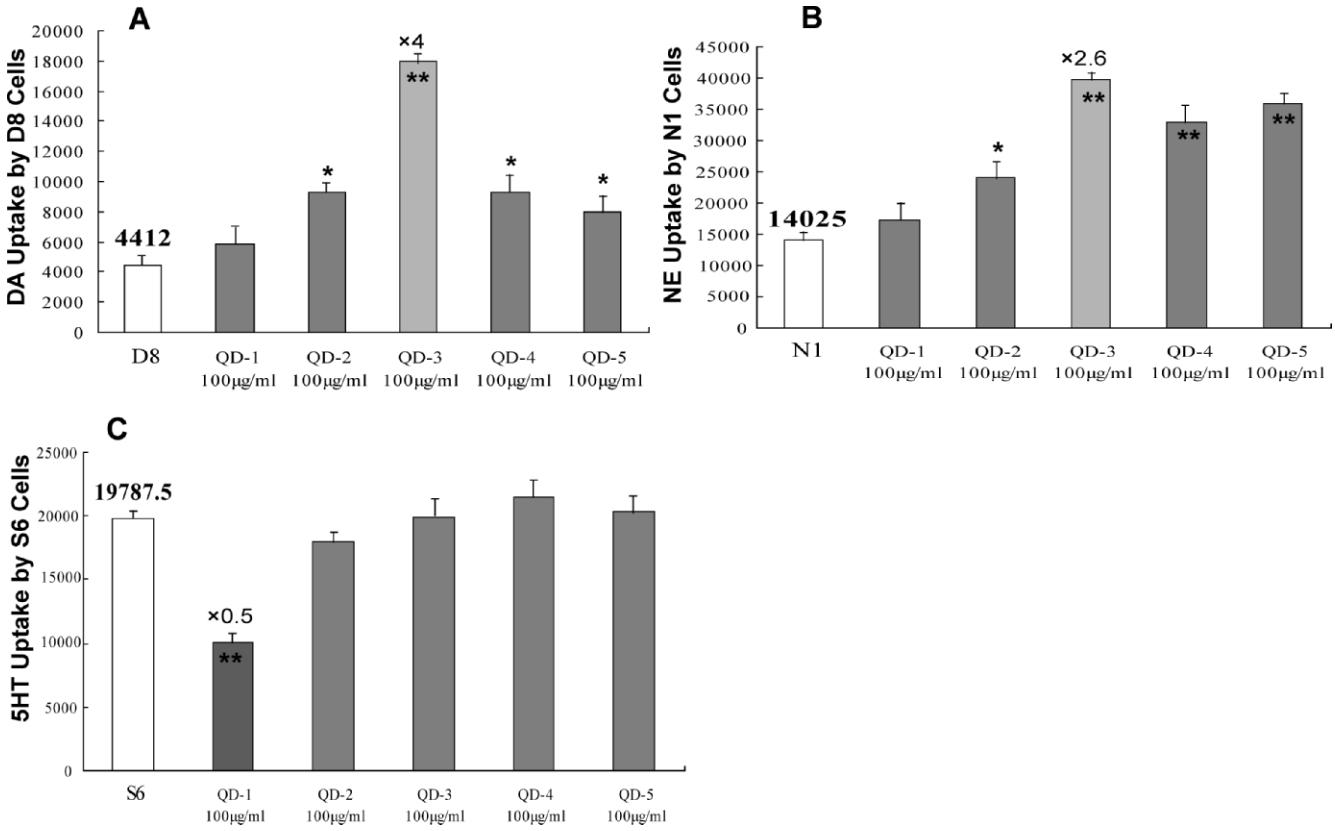

Figure 4. Effects of silica-gel fractions of QFT alkaloid-free chloroform extract (QD) on [^{3}H] labeled transmitter uptake in the Tr-CHO cells. Activity detection shows that, at concentrations indicated, the fractions QD-1, –2, –3, –4, and –5 increased DA uptake by D8 cells (A) and NE uptake by N1 cells (B), and QD-1 inhibited 5HT uptake by S6 cells (C). Y axis is expressed as DPM value, which is expressed as mean ± SEM of triplicate cell samples. * and **, $P < 0.05$ and 0.01, respectively, compared to corresponding control cell group.

tions of 5HT elicited an uptake curve typical of a saturation type, while co-incubation of QE-7 and QB-5 with 5HT produced a concentration-dependent downward shift of the curve (data not shown). The Michaelis-Menten constant (Km) and the maximal reuptake rate (Vmax) of the 5HT transport were generated using nonlinear regression (details seen in Materials and Methods) and then the two pharmacokinetic parameters obtained were expressed as column plots. As compared to vehicle control, QE-7 (10 and 30 μg/ml) and QB-5 (15 and 45 μg/ml) had no marked effects on Vmax of 5HT uptake (Fig. 8A), but the Km values were significantly increased in a concentration-dependent manner (Fig. 8B).

Discussion

Our lab utilized the neurotransmitter transporter-over-expressing CHO cells, i.e. D8, N1, S6, and G1 cells, to evaluate the potential effects of QFT extracts on neuro-transmitter transporters. Our initial results: at concentration of 100 μg/ml, QA, QC, QE, QD, and QB generally possessed enhancing actions on DA/NE uptake by D8 (Fig. 2B) or N1 cells (Fig. 2C), whereas QE or QB produced an inhibitory effect on 5HT uptake by S6 cells (Fig. 2D), and the five extracts had no clear action on GABA uptake in G1 cells (Fig. 2E). Further, of the three groups of fractions (QE-2, –3, –5, –7, QD-1, –2, –3, –4, –5, and QB-1, –2, –3, –4, –5) that were fractioned respectively from the primary candidate QE, QD, and QB extract, QE-2, –3, –5, and –7 possessed different degrees of enhancement of DA uptake (Fig. 3B) as well as inhibition of 5HT uptake (QE-2 also had a moderate enhancing action on NE uptake) (Fig. 3C and D), QD-1, –2, –3, –4, and –5 produced relatively lower potencies for not only enhancing DA/NE uptake (Fig. 4A and B) but also inhibiting 5HT uptake (Fig. 4C), and QB-1 and QB-5 had relatively even weaker bioactivities on DA/NE uptake enhancement and/or on monoamine uptake inhibition (Fig. 5A–C). The aforementioned facts demonstrate that QFT extracts can selectively regulate monoamine transports, actions that are actually typical of a dual regulation, as well as indicate that higher efficacies may be produced on the silica gel column chromatography.

Based on their excellent bioactivity (Fig. 3–5), the fraction QE-2, QE-7, QD-3, and QB-5 were adopted as candidates, for purpose of further corroboration of the uptake modulating effects as well as comparison of bioactivities over a broad concentration range (0.02 to 100 μg/ml). A clear concentration-effect relationship of drug acting by enhancing or inhibiting transmitter uptake was shown in cell group with QE-2, QE-7, QD-3, or QB-5 (Fig. 6), corroborating the primary finding concerning the uptake

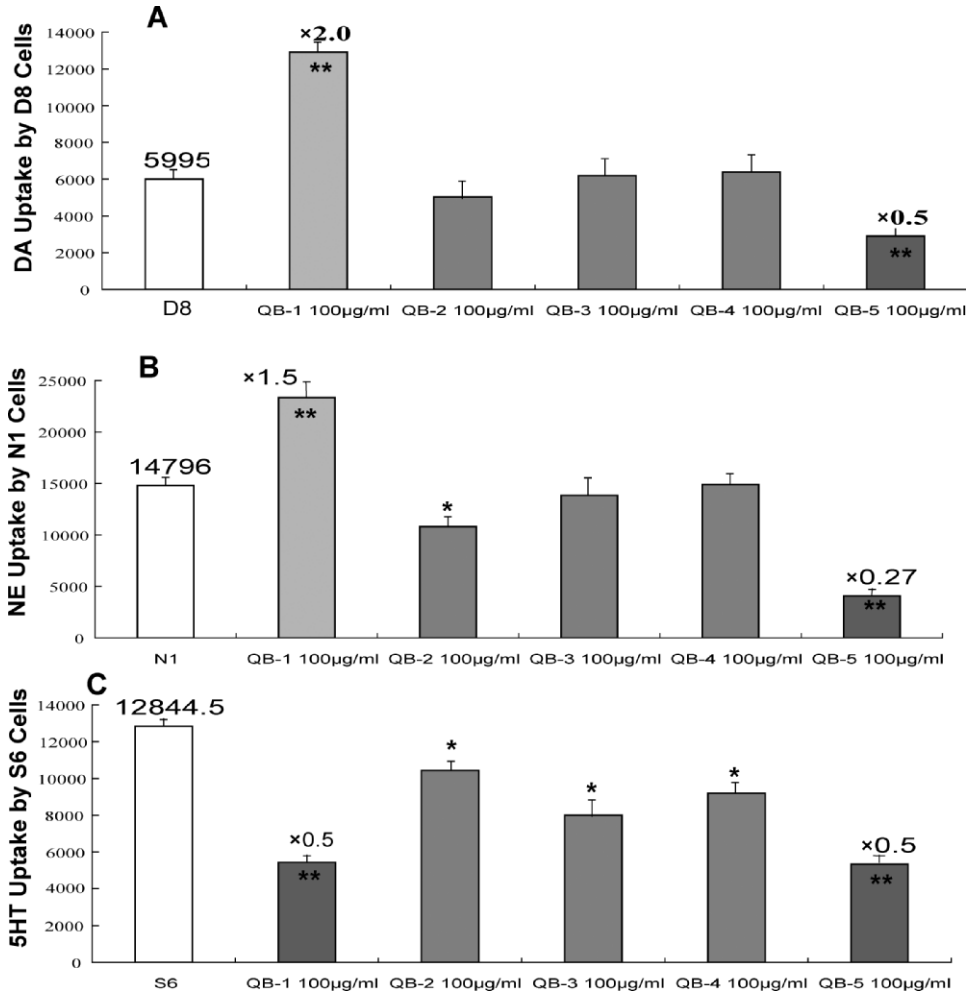

Figure 5. Effects of silica-gel fractions of QFT alkaloid-containing chloroform extract (QB) on [^{3}H] labeled transmitter uptake in the Tr-CHO cells. Activity detection shows that, at concentrations indicated, the fractions QB-1/-5 increased or inhibited DA uptake by D8 cells (A) and NE uptake by N1 cells (B), and QB-1/-5 inhibited 5HT uptake by S6 cells (C). Y axis is expressed as DPM value, which is expressed as mean ± SEM of triplicate cell samples. * and **, $P < 0.05$ and 0.01, respectively, compared to corresponding control cell group.

regulating actions produced by the testing drugs at concentration of 100 μg/ml. Further, QE-2 and QD-3 possess stronger potencies and higher efficacies for increasing DA uptake than for increasing NE uptake (Fig. 6A and C), indicating a selectivity to DA uptake; concerning the inhibition of monoamine uptake, QB-5 is, however, more selective for S6 system than for D8/N1 system (Fig. 6D). Furthermore, the rank order of potency or efficacy for enhancing DA/NE uptake is: QE-2 > QD-3 > QE-7 (QD-3 possesses an additional weak activation of 5HT uptake), and that for inhibiting 5HT uptake is: QE-7 > QB-5 (Fig. 6), indicating that some unknown active compounds may concentrate in the fraction of QE-2 or QE-7. Until now, various active constituents such as sinomenine, sinoacutine, disinomenine, and magnoflorine (19) were isolated from *Caulis Sinomenii*. Isolation of the trace chemicals hitting monoamine transporters may need to be considered in future study.

The aforementioned activation of monoamine transports suggests that QFT may be the potential activators of monoamine transporters. To test this hypothesis, we projected a protocol, in which the selective DAT inhibitor GBR12,935 or NET inhibitor desipramine was adopted and co-incubated with different concentrations of QE-2 or QD-3 in D8 or N1 uptake system. We showed that the enhanced uptake of DA/NE induced by corresponding QE-2 or QD-3 was markedly and concentration-dependently suppressed by GBR12,935 or desipramine (Fig. 7). Thus, the DA/NE-uptake-enhancing actions of QE-2 or QD-3 were dependent of the activity of DAT or NET, indicating that such functional activation occurs through a transporter targeting mechanism as well as demonstrating that both QE-2 and QD-3 may function as potent DAT/NET activators. In the CNS, monoamine mediates a wide array of physiological functions involved in behavior, cognition, and neuroendocrine secretion (20). A key step that determines the intensity and duration of monoamine signaling at synapses is reuptake of transmitter back into nerve terminals by the

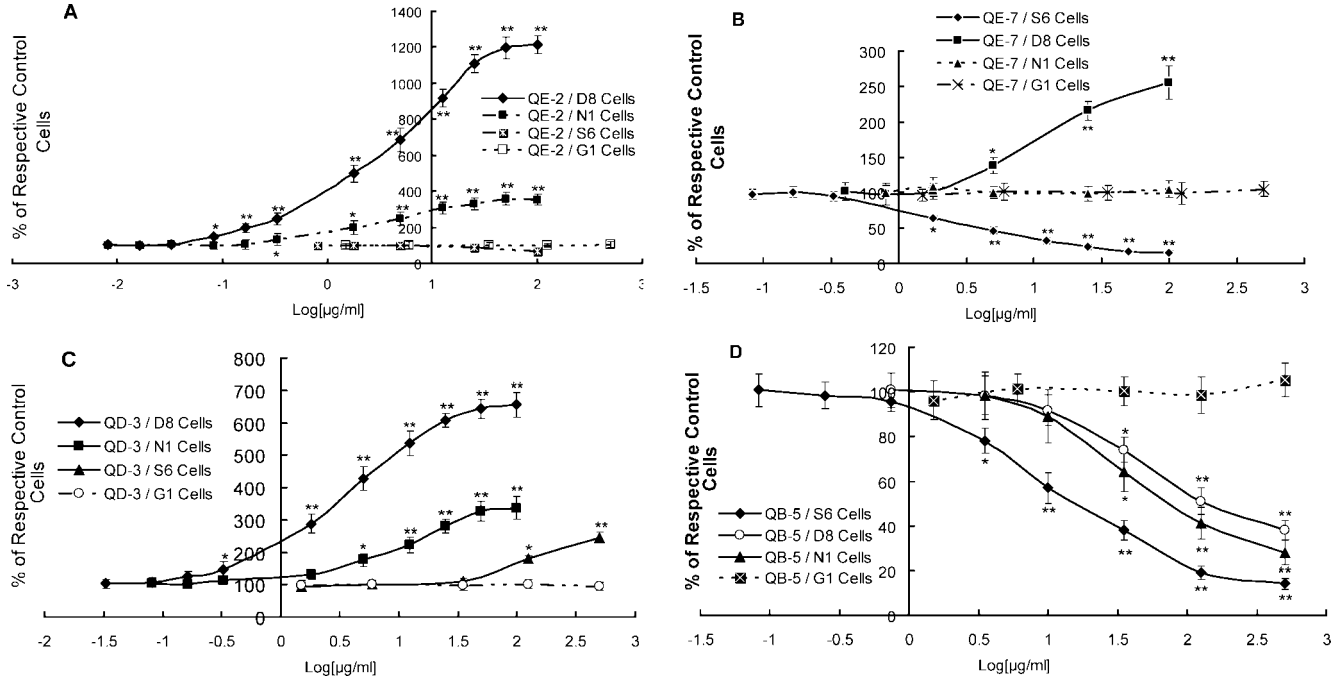

Figure 6. Semilog-concentration-response curve of QFT extracts for monoamine-uptake regulation. A. Activating effect of QE-2 on DA/NE-uptake by D8/N1 cells. B. Inhibitory or activating effect of QE-7 on 5HT or DA uptake by corresponding S6/D8 cells. C. Enhancing effects of QD-3 on DA/NE/5HT uptake by corresponding D8/N1/S6 cells. D. Inhibitory effects of QB-5 on 5HT/NE/DA uptake by corresponding S6/N1/D8 cells. * and **, $P < 0.05$ and 0.01, respectively, compared to corresponding control cell group. Value is expressed as mean ± SEM of triplicate cell samples.

plasma membrane transporters (21). DAT, functioning by limiting DA action at pre- and postsynaptic receptors and by maintaining normal DA homeostasis (20), plays a key role in regulating locomotion, emotion, and reward. Abnormal hyperfunction of dopaminergic system contributes to the development of several neurological and psychiatric conditions, such as schizophrenia (with positive symptoms) (22), anxiety disorder (23), obsessive-compulsive disorder (24), and drug addiction (9, 25, 26). NET, another member of monoamine transporter family, mainly confined to the hippocampus and cortex, mediates norepinephrinergic signaling which is also involved in emotion and drug dependence (10). QFT extracts, especially QE-2 and QD-3, as novel dual DAT/NET activators as shown in the current study, are thus supposed to possess a possible antipsychotic or anti-addictive action. Previous literatures have reported that QFT or its isolates possess analgesic, sedative, hypotensive, and histamine-releasing actions (27, 28) as well as an anxiolytic-like action (29), and more recently, several preclinical studies have shown that one of its isolates can decrease the degree of preference to morphine (8). These aforementioned facts concerning the action profile of QFT could be explained by its functional enhancement of DAT/NET and, together with our current findings, support the hypothesis that the functional enhancement of DAT/NET by QFT extracts (especially QE-2 and QD-3) could contribute to various sedative and antipsychotic effects.

Pharmacokinetic mechanism of the inhibitory actions of QE-7 and QB-5 on 5HT uptake was explored using the S6 system for the purpose of confirming its SERT-targeting effect. Our current result showed that both QE-7 and QB-5 concentration-dependently decreased the Km values of 5HT uptake, but did not affect its Vmax value (Fig. 8). Thus, the 5HT-uptake inhibition by the two fractions was through a competitive mechanism or direct SERT-targeting mechanism. SERT, another member of the Na^+/Cl^--coupled transporter family, is the cellular binding site for several clinically used antidepressants acting by acutely increasing extracellular 5HT levels (30); and this protein, considered to be involved in the pathogenesis of affective disorders, is seen as a target site for screening novel antidepressants (31), with its antagonists at the forefront of current drug development. Additionally, preliminary data from animal and clinic studies showed that 5HT re-uptake inhibitors also are promising in motor recovery after stroke (31, 32). Therefore, QE-7 and QB-5 as potent SERT inhibitors could possess antidepressant action or would be helpful in stroke recovery.

In summary, the present pharmacological study demonstrated that QFT extracts are novel monoamine transporter modulators, wherein QE-2, QD-3, and QE-7 are potent DAT/NET activators, while QE-7 and QB-5 are potent SERT inhibitors. QFT extracts, especially QE-2 and QE-7, both with stronger potencies, therefore, could be effective for monoamine-associated neuropsychological disorders (e.g. major depression, bipolar disorder, schizophrenia, and drug problems). For further study, the active compounds will be isolated and identified from QFT

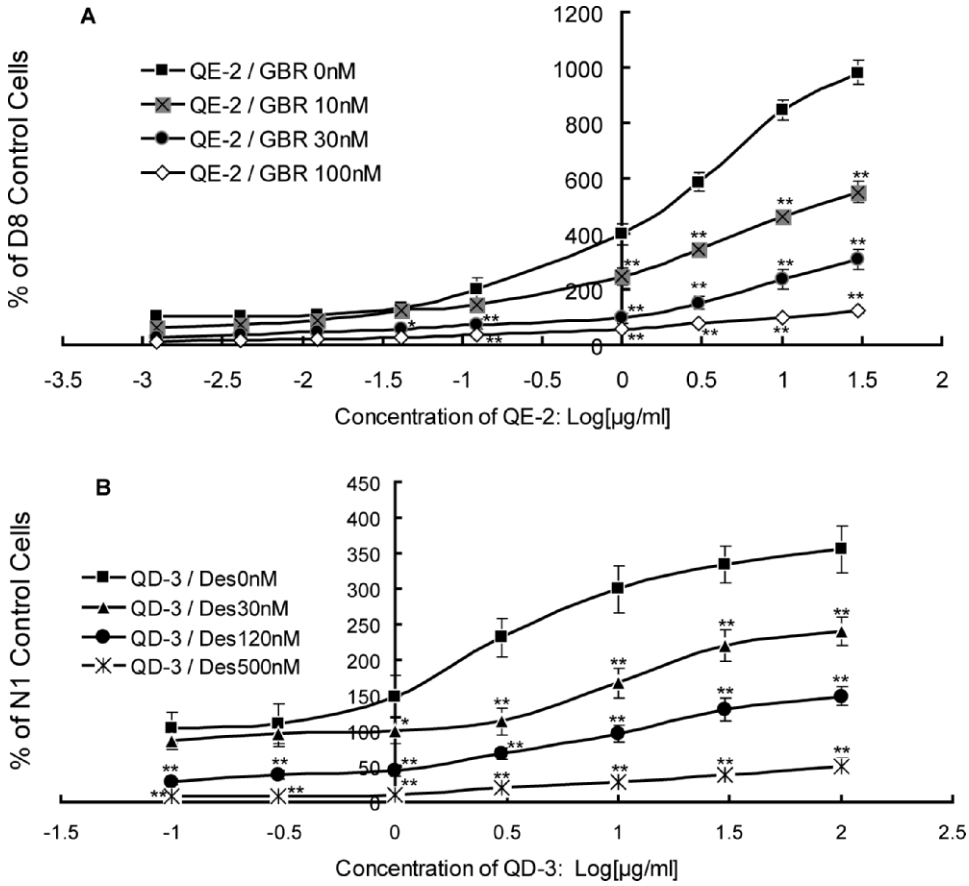

Figure 7. Effects of the selective DAT/NET reference inhibitors on QFT extract-induced increases of DA/NE uptake. A. GBR12,935 (GBR) concentration-dependently reduced the degree of DA-uptake enhancement by QE-2 in D8 cells. B. Desipramine (Des) concentration-dependently inhibited the degree of NE-uptake enhancement by QD-3 in N1 cells. * and **, $P < 0.05$ and 0.01, respectively, compared to corresponding control cell group. Values are expressed as mean ± SEM of triplicate cell samples.

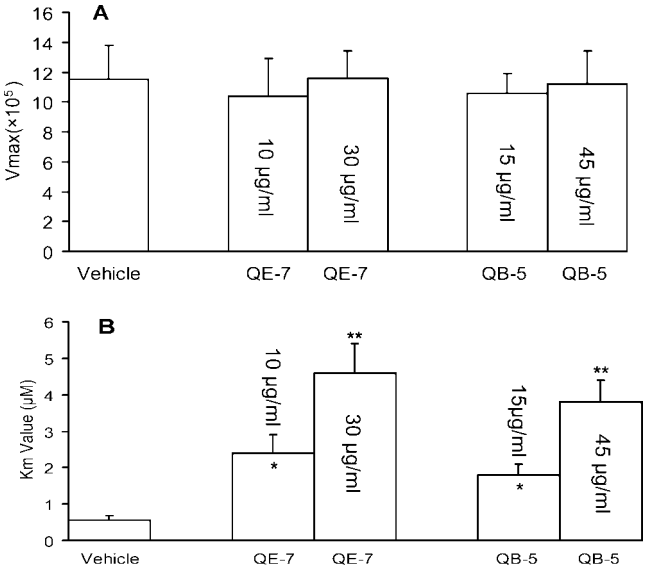

Figure 8. Pharmacokinetic mechanism of 5HT-uptake inhibition by both QE-7 and QB-5. A. Effects of QE-7 (10 and 30 μg/ml) and QB-5 (15 and 45 μg/ml) on Vmax of 5HT uptake. B. Effects of QE-7 and QB-5 on Km value of 5HT uptake. * and **, $P < 0.05$ and 0.01, respectively, compared to vehicle. Values are expressed as mean ± SEM of three experiments in quintuplicate S6 cell samples.

extracts (especially QE-2 and QE-7), and may be structurally modified as potentially more robust lead compounds. Because, until now, there is no practical pharmaceutical that can act as agonist of neurotransmitter transporters, identification of novel DAT/NET activator(s) from the herbal material QFT would be profoundly meaningful in both clinical medicine and the academic field.

We thank Mr. Jie Wang for help in cell culture. We also thank Prof. Jian Fei for direction in neuropharmacology. All the authors are employees of Cell Star Bio-Technologies Co., Limited and/or Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and have no conflict of interest.

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