Current Medicinal Chemistry, 2018, *25*, 1036-1045

REVIEW ARTICLE

Serotonin Receptor Binding Characteristics of Geissoschizine Methyl Ether, an Indole Alkaloid in Uncaria Hook

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Abstract: ***Background:*** Geissoschizine methyl ether (GM) is one of the indole alkaloids in Uncaria hook, and an active ingredient of yokukansan (YKS) that improves behavioral and psychological symptoms of dementia (BPSD) in patients with several types of dementia. The pharmacological action of GM has been related to various serotonin (5-HT) receptor subtypes.

Objective: The aim of this article is to review the binding characteristics of GM to the 5-HT receptor subtypes in the brains using our own data and previous findings.

Method: Competitive receptor-binding and agonist/antagonist activity assays for several 5-HT receptor subtypes were performed. Moreover, the articles describing pharmacokinetics and brain distribution of GM were searched in PubMed.

Results: GM bound the following 5-HT receptor subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_4, 5-HT_{5A}, 5-HT_6, and 5-HT_7. Among these receptors, GM had partial agonistic activity for 5-HT_{1A} receptors and antagonistic activity for 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_7 receptors. Also, GM was metabolized by various CYP isoforms, mainly CYP3A4. Parent/unchanged GM was detected in both the blood and brain of rats after oral administration of YKS. In the brains, GM was presumed to bind to 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_7 receptors on neuron-like large cells mainly in the frontal cortex.

Conclusion: These results suggest that GM is a pharmacologically important alkaloid that regulates various serotonergic activities or functions by binding to multiple 5-HT receptor subtypes. Thus, this review provides recent 5-HT receptor-related evidence that GM is partly responsible for pharmacological effects of YKS.

ARTICLE HISTORY

Received: January 16, 2017
Revised: March 08, 2017
Accepted: March 15, 2017

DOI:
10.2174/0929867324666170320114713

Keywords: Geissoschizine methyl ether, 5-HT receptor, pharmacokinetics, pharmacological aspect, yokukansan, BPSD, dementia.

1. INTRODUCTION

Yokukansan (YKS) is one of the traditional Japanese medicines called Kampo medicines in Japan, and has been approved by the Japanese Ministry of Health, Labour, and Welfare as a remedy for neurosis, insomnia, and irritability and night crying in children. YKS reportedly improves behavioral and psychological symptoms of dementia (BPSD) such as hallucinations, agitation, and aggressiveness in patients with different types of dementia, including Alzheimer's disease [1-4], dementia with Lewy bodies [5], vascular dementia [6], and frontotemporal dementia [7], without severe adverse effects.

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Accumulated basic research has demonstrated that the serotonergic system in the central nervous system (CNS) plays an important role in the psychotropic effects of YKS [8-10]. An *in vitro* binding study showed that YKS bound to a serotonin 1A (5-HT_{1A}) receptor as a partial agonist [9]. A subsequent study clarified that only Uncaria hook, among seven constituent medicinal herbs of YKS, had the partial agonistic activity to 5-HT_{1A} receptor [9]. This finding was also verified by the evidence that the partial agonistic binding of YKS disappeared after removing Uncaria hook from YKS. These results imply that the active ingredients showing 5-HT_{1A} receptor partial agonistic activity are contained in Uncaria hook. Further *in vitro* receptor-binding assay identified geissoschizine methyl ether (GM) as the active ingredient, which is an indole alkaloid in Uncaria plants [11,12] (Fig. **1**).

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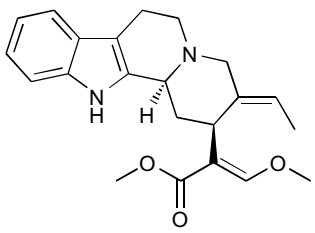


Fig. (1). Chemical structure of geissoschizine methyl ether (GM).

These *in vitro* findings were supported by *in vivo* studies using rodents, which have demonstrated that oral YKS (1.0 g/kg) ameliorated BPSD-like aggressive and social behaviors and that these ameliorative effects were counteracted by a 5-HT_{1A} receptor antagonist [8,10]. This finding was also verified by the study that the ameliorative effect of YKS on isolation stress-induced aggressive behavior was completely abolished by the removal of Uncaria hook, suggesting that the effect of YKS is mainly attributed to Uncaria hook [10]. Moreover, Uncaria hook alone (150 mg/kg, the approximate amount of Uncaria hook contained in 1.0 g/kg of YKS) or GM alone (150 μg/kg, the approximate amount of GM contained in 1.0 g/kg of YKS) also ameliorated isolation stress-induced aggressive behavior, which had similar efficacy to YKS [10]. Pharmacokinetic study demonstrated that GM was detected in the plasma and brain of rats after oral administration of YKS [13,14]. These results suggest that GM is a potent 5-HT_{1A} receptor agonist and a candidate ingredient for the psychopharmacological effect of YKS.

GM has an indole structure similar to that of the neurotransmitter 5-HT. 5-HT receptors that are instrumental in various physiological functions are known to have at least 14 subtypes from seven distinct families (5-HT_1–5-HT_7) [15]. Therefore, GM might mediate multiple serotonergic physiological functions via several 5-HT receptor subtypes. Indeed, to date, GM has demonstrated binding ability to several subtypes of 5-HT receptor [10,16-21]. In this review, we describe our data indicating the binding profile and agonist/antagonist activity of GM for various 5-HT receptor subtypes, with reference to previous findings. The pharmacokinetics and pharmacological aspects of GM and YKS are also described. These findings provide druggable information of a natural compound GM, and would be useful in understanding the contribution of GM to the pharmacological effects of YKS.

2. ISOLATION AND IDENTIFICATION OF GM

We isolated GM from Uncaria hook, *i.e.* the hook of *Uncaria rhynchophilla* Miquel, Rubiaceae [22]. In brief, 319.4 g of a dried crude drug of Uncaria hook dissolved in 2.5 L of distilled water was refluxed at 120°C for 2 h. The extracted solution was passed through a 100 mesh-size stainless steel filter and then lyophilized to give a dried powder (38.2 g). The extract was chromatographed on a Diaion HP-20 (Mitsubishi, Tokyo, Japan), eluted with 2 L of water, 2 L of aqueous methanol (50% v/v), and 1 L of methanol, successively. The methanol eluate was evaporated to remove the solvent and then lyophilized to afford the dried methanol–eluate powder (0.529 g). The indole alkaloids were further isolated from the methanol extract by eluting with 0.05 M ammonium acetate buffer (pH 3.6)–acetonitrile (1:1) on a separation column (ODS, 5 cm i.d. × 30 cm, Inertsil, GL Science, Tokyo, Japan), yielding 10 mg of GM. In direct comparison with an authentic standard substance, the isolated GM was confirmed to be a single peak by high performance liquid chromatography, and was identified by analyses of the ^{1}H and ^{13}C nuclear magnetic resonance spectra and mass spectrum.

3. RECEPTOR BINDING

This section introduces the foundational data regarding the binding of GM on 5-HT receptor subtypes. Competitive binding assays for 5-HT_{1A} [23, 24], 5-HT_{1B} [25, 26], 5-HT_{2A} [27, 28], 5-HT_{2B} [27], 5-HT_{2C} [29], 5-HT_3 [30, 31], 5-HT_4 [32], 5-HT_{5A} [33], 5-HT_6 [34], and 5-HT_7 [35, 36] were performed according to the previously reported procedures. The membrane preparations of Chinese hamster ovary (CHO) cells stably expressing human recombinant 5-HT_{1A} and 5-HT_7, CHO-K1 cells stably expressing human recombinant 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{5A}, human embryonic kidney (HEK)-293 cells stably expressing human recombinant 5-HT_3, and Hela cells stably expressing human recombinant 5-HT_6, were used for the respective corresponding binding assays. Membrane preparations of rat cerebral cortex and guinea pig striatum were used for the binding assays of 5-HT_{1B} and 5-HT_4 receptors. Radioligands used for each receptor assay were [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) for 5-HT_{1A}; [^{125}I]cyanopindiolol for 5-HT_{1B}; [^{3}H]ketanserin for 5-HT_{2A}; [^{3}H]lysergic acid diethylamide for 5-HT_{2B}, 5-HT_{5A}, 5-HT_6, and 5-HT_7; [^{3}H]mesulergine for 5-HT_{2C}; [^{3}H]GR-65630 for 5-HT_3; and [^{3}H]GR-113808 for 5-HT_4. Metergoline (5-HT_{1A}), serotonin (5-HT_{1B}, 5-HT_{2B}, 5-HT_4, 5-HT_{5A}, 5-HT_6, and 5-HT_7), mianserin (5-HT_{2A} and 5-HT_{2C}), and MDL 72222 (5-HT_3) were used to determine the nonspecific binding for each receptor. The binding specificities of these binding assay procedures were approximately

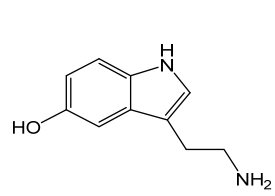
75%–95%. In these assays, metergoline, 5-HT, ketanserin, SB242084, MDL 72222, RS-23595-190, and methiothepin were used as the reference compounds (Fig. **2**).

Fig. (**3**) shows the concentration-response curves to determine the half maximal inhibitory concentration (IC_{50}) values of GM to each 5-HT receptor subtype in the competitive binding assays. The sigmoidal curve for each reference compound indicated that the binding assays used in this study were appropriated to evaluate the binding of test substances. GM strongly inhibited the radioligand bindings to 5-HT_{1A} (IC_{50} = 0.904 μM), 5-HT_{2A} (IC_{50} = 0.197 μM), 5-HT_{2B} (IC_{50} = 0.191 μM), 5-HT_{2C} (IC_{50} = 1.480 μM), and 5-HT_7 (IC_{50} = 0.034 μM) receptors rather than other subtypes of 5-HT_{1B} (IC_{50} = 88.3 μM), 5-HT_3 (non-binding), 5-HT_4 (IC_{50} = 94.5 μM), 5-HT_{5A} (IC_{50} = 6.84 μM), and 5-HT_6 (IC_{50} = 12.2 μM). The results suggest that GM bound to 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_7 receptor subtypes.

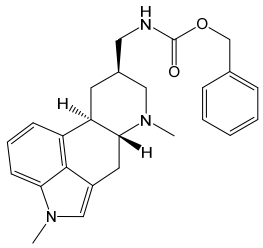
Kanatani *et al.* [16] reported that GM inhibited specific [^{3}H]5-HT binding to rat brain membrane; however, they did not determine the target receptor subtype. Thereafter, Pengsuparp *et al.* [18] demonstrated that GM inhibited the specific binding of [^{3}H]radioligands for 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors to mouse brain membrane. Our competitive binding assays using radioligands demonstrated that GM shows more potently binds to not only 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} but also 5-HT_{2B} and 5-HT_7 receptors in various cells expressing each human recombinant 5-HT receptor subtype.

4. AGONIST AND ANTAGONIST ACTIVITIES

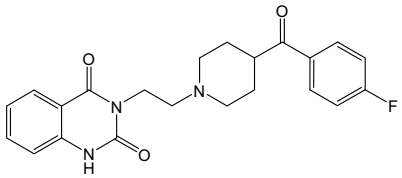
Subsequently, we examined whether GM shows agonistic or antagonistic activity to five 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2c}, and 5-HT_7) that GM showed potent binding in the competitive receptor-binding assays. The agonistic effects of GM were evaluated by measuring [^{35}S]GTPγS binding for 5-HT_{1A} [37] and 5-HT_{2C} [37, 38] receptors, inositol



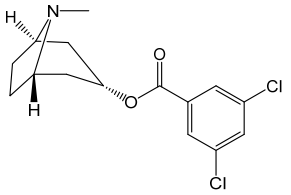
Serotonin (5-HT)



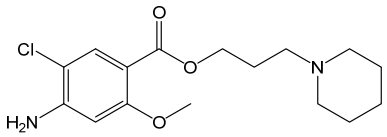
Metergoline



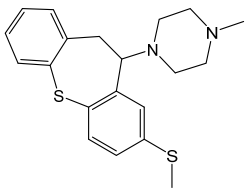
Ketanserin



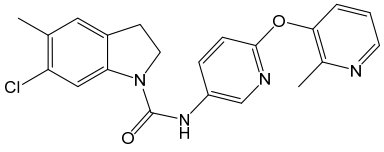
MDL-72222



RS 23597-190



Methiothepin



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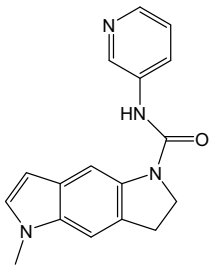

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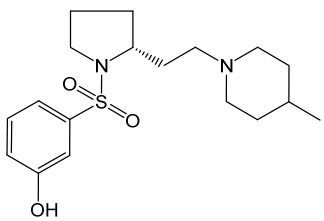

SB269970

Fig. (2). Chemical structures of the reference compounds used in the binding and agonist/antagonist assays to 5-HT receptor subtypes.

monophosphate (IP_1) for 5-HT_{2A} [39, 40] and 5-HT_{2B} [41,42] receptors, or cAMP for 5-HT_7 receptors [43] in the cells expressing each receptor subtype. The antagonistic effects of GM on these receptors were assessed by examining the inhibition of 5-HT-induced increases in [^{35}S]GTPγS binding, IP_1 production, or cAMP production. In these assays, metergoline, 5-HT, ketanserin, SB242084, MDL 72222, RS-23595-190, and methiothepin were used as the reference compounds (Fig. **2**).

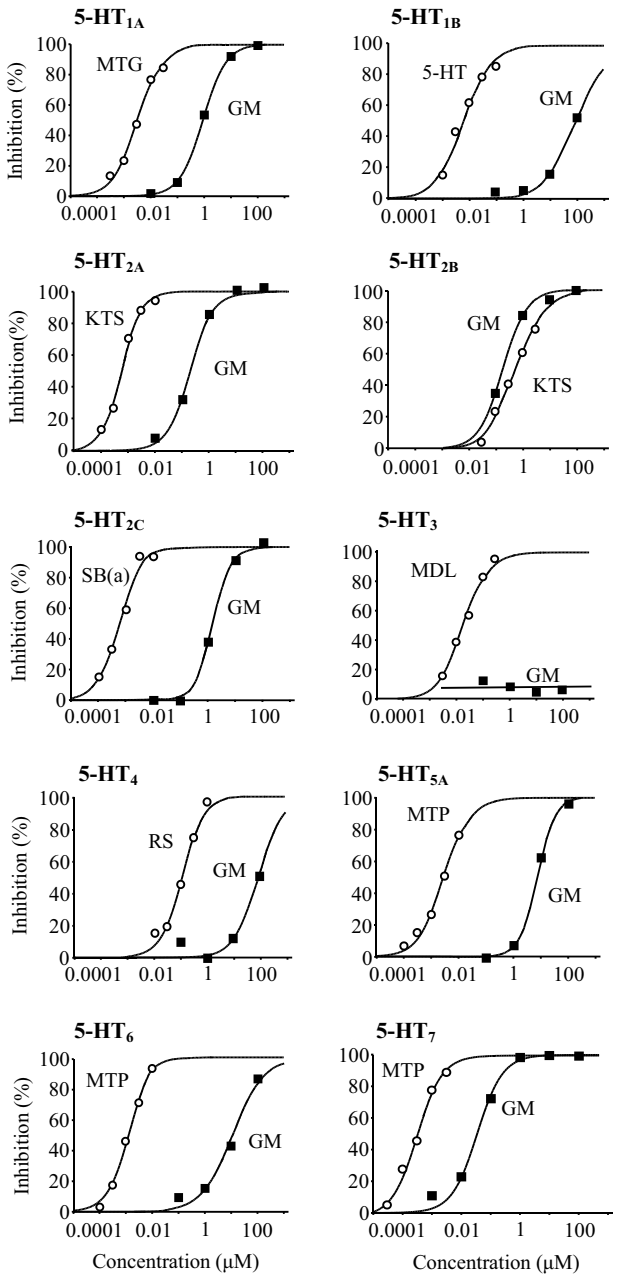

Fig. (3). The concentration–response curves of GM and reference compounds to 5-HT receptor subtypes in the competitive binding assays. Each data represents the mean of duplicate determinations. MTG, metergoline; KTS, ketanserin; SB(a), SB242084; MDL, MDL 72222; RS, RS-23597-190; MTP, methiothepin.

Fig. (**4**) shows the concentration-response curves of GM and reference compounds to each receptor. Agonistic activity was found in 5-HT_{1A} receptors: the [^{35}S]GTPγS binding was increased by GM or 5-HT, a full agonist, in a concentration-dependent manner. However, the binding rate of GM reached a plateau at approximately 40% of that of 5-HT, suggesting that GM is a partial agonist for 5-HT_{1A} receptor [10]. Regarding the four other receptors, GM showed antagonistic activity with IC_{50} values of 2.31 μM (5-HT_{2A}), 0.182 μM (5-HT_{2B}), 6.19 μM (5-HT_{2C}), and 6.00 μM (5-HT_7).

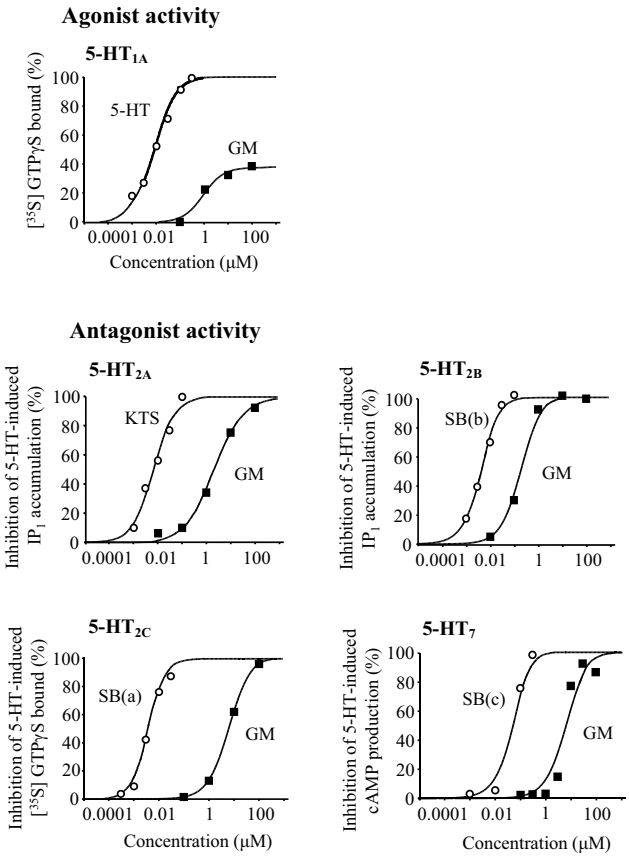

Fig. (4). Agonist and antagonist activities of GM. GM showed partial agonistic activity for 5-HT_{1A} receptor and antagonistic activity for 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_7 receptors. Each data represents the mean of duplicate determinations. KTS, ketanserin, SB(a), SB242084; SB(b), SB206553; SB(c), SB269970.

GM was initially found by Kanatani *et al.* [16] to have partial agonistic activity for 5-HT receptors in a combination of [^{3}H]5-HT-binding assay of rat brain

membrane and bioassay using guinea-pig ileum, but they did not determine the receptor subtypes. Meanwhile, Zhu *et al.* [17] reported that Uncaria hook exhibited strong binding to 5-HT_{1A} and 5-HT_2 receptors, but they did not determine the active ingredient. In 2001, Pengsuparp *et al.* [18] reported that GM possessed mixed 5-HT_{1A} receptor agonist/5-$HT_{2A/2C}$ receptor antagonist activities by using various bioassays such as hypothermic response, head-twitch response, and head-weaving response. Recently, Ueda *et al.* [19] verified them in another analytical approach, *i.e.,* a single-cell-based calcium imaging assay using HEK-293T cells expressing each human recombinant 5-HT receptor subtype. From these findings and our results (Figs. **3** and **4**), it is no doubt that GM possesses 5-HT_{1A} receptor partial agonist and 5-$HT_{2A/2C}$ receptor antagonist activities.

We also found that GM possessed antagonistic activity to the 5-HT_{2B} receptor. GM contains tetrahydro-β-carboline (THBC) in its structure, which has been reported to show selective antagonist activity on the 5-HT_{2B} receptor [44, 45]. Rauwolscine is also an indole alkaloid containing the THBC structure, and is reported to behave as a 5-HT_{1A} receptor partial agonist, a 5-$HT_{2A/2B}$ receptor antagonist [46-48], as well as an α_2-adrenergic receptor antagonist [49, 50]. It is suggested that the presence of the D-ring and the substituents of THBC [18], in other words, the C1-substituted optically activity of THBC [51], increases the affinity for 5-HT receptor subtypes. Since GM is also a C1-substituted THBC, GM is thought to have 5-HT_{2B} antagonistic activity.

Regarding the 5-HT_7 receptor, Ueda *et al.* [19] first demonstrated that GM behaved as the antagonist in addition to 5-HT_{1A} partial agonist, 5-$HT_{2A/2C}$ antagonist, and a D_{2L} receptor partial agonist/antagonist in a single-cell-based calcium imaging assay. The 5-HT_7 receptor is a G protein-coupled receptor linked to $G\alpha_s$ that activates adenylate cyclase, and increases second messenger cAMP [52]. Because this receptor does not link to an intracellular calcium mobilization ($[Ca^{2+}]_i$) system, it is different from $G\alpha_q$-linked G protein-coupled receptor-like 5-HT_2 receptors, which activate inositol trisphosphate, and then induce $[Ca^{2+}]_i$ mobilization [53]. Thus, although it is generally impossible to evaluate the intrinsic activity of 5-HT_7 receptors by changes in $[Ca^{2+}]_i$ mobilization, the calcium imaging assay newly developed by Ueda *et al.* [54] enabled it by transfection of $G\alpha_{15}$ ($G\alpha_{15}$ integrates into the downstream calcium flux) in HEK-293T cells expressing human recombinant 5-HT_7 receptors. However, the receptor binding rate of the test substance is not clear in this method, and measurement of the cAMP level is the most appropriate for the G protein-coupled receptors linked to $G\alpha_s$ and $G\alpha_i$ for direct and absolute evaluation. Our present data support these issues by clarifying the antagonistic effect of GM on 5-HT_7 receptor using competitive binding assay (Fig. **3**, 5-HT_7) and direct measurement of intracellular cAMP levels (Fig. **4**, 5-HT_7). In our 5-HT_7 receptor assay [20], only GM, among the seven alkaloids in Uncaria hook (indole alkaloids: GM, hirsuteine, and hirsutine; oxindole alkaloids: rhynchophylline, isorhynchophylline, corynoxeine, and isocorynoxeine), showed 5-HT_7 receptor antagonistic activity. Structural comparison of these ingredients inferred that the binding to 5-HT_7 receptor also depends on the difference of optical isomer at the C1-substituent in the THBC structure [51], as described above.

Several chemical compounds with 5-HT_7 receptor antagonistic activity reportedly also have 5-HT_1 agonist and 5-HT_2 receptor antagonist activities [55-57]. As already described, GM has an agonistic effect on 5HT_{1A} receptors, and an antagonistic effect on 5-HT_{2A} receptors. These findings also support that GM has a high affinity for 5-HT_7 receptors.

5. PHARMACOKINETICS

In vitro studies using rat and human liver microsomes reported that GM was metabolized into at least 13 metabolites including hydroxylated, dehydrogenated, hydroxylated + dehydrogenated, demethylated, and hydration forms by several CYP isoforms, and CYP3A4 was found to mainly contribute to GM metabolism [58, 59]. Parent/unchanged GM was detected in both plasma and brain of rats after orally administered YKS, and demonstrated that GM was able to cross the blood-brain barrier (BBB) in an *in vitro* BBB assay [13,14]. Recently, Kitagawa *et al.* [60] verified GM to be detected in the plasma after oral administration of YKS in humans. These *in vivo* and *in vitro* results suggest that GM in orally administered YKS is absorbed into the blood, and then reaches the brain through the BBB. The GM that entered the brain was presumed to bind to dopamine D_2, adrenergic α_{2A}, and μ-opioid receptors and L-type Ca^{2+} channels, as well as 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_7 receptors on neuron-like large cells mainly in the frontal cortex, which were evaluated by autoradiography using [^{3}H]GM in rat brain slices [21]. This result agrees with those in previous *in vitro* binding assays [10,18-20].

Pharmacokinetics of GM metabolites identified in the *in vitro* study has not yet been verified *in vivo* study. However, we confirmed in a preliminary study that 23-*O*-demethylated GM was also detected in the brain of YKS-treated rats, and *in vitro* receptor binding assay showed that this metabolite did not bind to the 5-HT_{1A} receptors (unpublished observations). These results suggest that GM but not the metabolite is the active form.

6. PHARMACOLOGICAL ASPECT

In humans, 90% of the 5-HT in the body exists in the gastrointestinal tract, 8%–10% in platelets, and 1%–2% in the CNS. In the peripheral nervous system, 5-HT is involved in smooth muscle contraction, gastrointestinal function, and platelet aggregation. In the CNS as a neurotransmitter, it is related to physiological functions such as mood and emotional regulation, sleep-wake cycle, thermoregulation, sexual behavior, algesia, cognition/memory formation, and biorhythm. Dysfunction of the serotonergic system is involved in various mental disorders such as anxiety, aggressiveness, duress, mood disorders, schizophrenia, autism, and drug dependence [61]. Complex natural alkaloids that contain the THBC structure such as yohimbine or reserpine have a wide range of pharmacological activities. These types of molecule are known to have 5-HT receptor antagonist and α-adrenergic receptor antagonist activity, and have a broad spectrum of pharmacological properties including central action related to hallucination, vasodilation, and analgesic actions, as well as antimicrobial activities [44, 51, 62]. YKS containing GM, one of the THBCs, has various pharmacological effects that act to improve symptoms that are similar to BPSDs, like aggressiveness, hallucinations, anxiety, and sleep disturbance, as well as symptoms like tardive dyskinesia, neuropathic pain, morphine tolerance/physical dependency, allergy/atopic dermatitis, and cognitive deficits [63]. These multiple potential actions include serotonergic, glutamatergic, cholinergic, dopaminergic, adrenergic, and GABAnergic neurotransmissions as well as neuroprotection, anti-stress effect, promotion of neuroplasticity, and anti-inflammatory effect [63]. Among these neuropsychopharmacological effects, YKS, Uncaria hook, or GM has been demonstrated to enhance 5-HT_{1A} receptor agonist-induced decrease in rearing behavior, concomitant with up-regulation of prefrontal 5-HT_{1A} receptors in mice [64], or to ameliorate aggressiveness and decreased sociability [10] in isolation-stressed mice, anxiety in fear-conditioned rats [65, 66] through their agonistic effect to 5-HT_{1A} receptors [10], 5-hydroxy-L-tryptophan-induced head-twitch response which are related to 5-HT_{2A} and 5-HT_{2C} receptor antagonisms [18], and 5-HT_{2A} receptor agonist-induced head-twitch response by down-regulating 5-HT_{2A} receptors in the prefrontal cortex [67, 68]. In addition, these substances act on other neurotransmitter systems to improve symptoms, *e.g.,* adrenergic/dopaminergic agonist-induced decrease in locomotion [18, 69], morphine-induced tolerance/physical dependency in mice by blocking α_{2A}-adrenoceptors [70], norepinephrine-induced contraction of rat aorta [22], and glutamate-induced neuronal death [71].

Although the physiological functions of 5-HT_7 receptor are not fully understood, several studies suggest an involvement in vascular relaxation [36, 72] and circadian rhythm control [73, 74]. Hedlund and Sutcliffe [75] also suggest important functional roles for the 5-HT_7 receptor in thermoregulation, circadian rhythm, learning and memory, hippocampal signaling, and sleep. In addition, because atypical antipsychotics, such as clozapine and risperidone, and some antidepressants display high affinity for the 5-HT_7 receptor as antagonists, blocking effects of this receptor by these drugs are involved in antipsychotic or antidepressant action [57, 76, 77]. Ueda *et al.* [19] suggest that the pharmacological profiles of GM at dopamine and serotonin receptors are similar to those of aripiprazole, a third-generation antipsychotic. As described above, GM having 5-HT_7 receptor antagonist activity (Figs. **3** and **4**) was actually demonstrated to have anti-aggressive and vasorelaxant effects. YKS also has an ameliorative effect on rapid eye movement sleep behavior disorder in humans [78], which is related to circadian rhythm control. Thus, 5-HT_7 receptor antagonism is thought to relate to the psychotropic and vasorelaxant effects of GM and YKS.

Recently, Deng *et al.* [79] reported that several ingredients in *Angelica sinensis* exhibited affinity toward 5-HT_7 receptors in the *in vitro* competitive binding assay. Ofir *et al.* [80] reported that several isoflavans isolated from the roots of *Glycyrrhiza glabra* inhibited *in vitro* serotonin re-uptake. Although these herbal medicines differ from those included in YKS in the botanical origin; *Angelica acutiloba* and *Glycyrrhiza uralensis* are used in YKS, they are also informative for drug discovery and development for serotonin receptors in future.

CONCLUSION

This review provided 5-HT receptor-related evidence of GM responsible for pharmacological effects

of YKS. GM is thought to be a pharmacologically important alkaloid in regulating various serotonergic activities or functions by binding multiple 5-HT receptor subtypes. We hope this review forms the foundation for assessing the usefulness of natural compounds on neurotransmitter systems in the CNS.

LIST OF ABBRIBIATIONS

5-HT	=	5-Hydroxytriptamine (serotonin)
8-OH-DPAT	=	8-Hydroxy-2-(di-n-propylamino)tetralin
BBB	=	Blood-brain barrier
BPSD	=	Behavioral and psychological symptoms of dementia
cAMP	=	Cyclic adenosine 3',5'-monophosphate
CHO	=	Chinese hamster ovary
CNS	=	Central nervous system
CYP	=	Cytochrome P450
GABA	=	Gamma-aminobutyric acid
GM	=	Geissoschizine methyl ether
GTPγS	=	Guanosine 5'-O-(3-thiotriphosphate)
HEK	=	Human embryonic kidney
IC_{50}	=	Half maximal inhibitory concentration
KTS	=	Ketanserin
MDL	=	MDL 72222
MTG	=	metergoline
MTP	=	Methiothepin
RS	=	RS-23597-190
SB(a)	=	SB206553
SB(b)	=	SB242084
SB(c)	=	SB269970.
THBC	=	Tetrahydro-β-carboline
YKS	=	Yokukansan

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors are employees of Tsumura & Co. The authors declare that except for income received from the employer, no financial support or compensation has been received from any individual or corporate entity and no conflict of interest exists.

ACKNOWLEDGEMENTS

For studies conducted at Tsumura Research Laboratories, the authors thank the following researchers and managers for planning the studies, performing the experiments, analyzing the data, writing the papers, and stimulating discussion: Toshiyuki Ueki, Zenji Kawakami, Masahiro Tabuchi, Sachiko Imamura, Akinori Nishi, Hitomi Kanno, Takuji Yamaguchi, Hiroaki Ooizumi, Nae Kuriyama, Takashi Matsumoto, Hirotaka Kushida, Junko Watanabe, Tomohisa Hattori, and Yoshio Kase.

The authors would like to thank Enago (www.enago.jp) for the English language review.

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