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5-HT_3 Receptor Blocking Activity of Arylalkanes Isolated from the Rhizome of *Zingiber officinale*

Abstract

Different extracts (ethanolic, hexane, aqueous) of ginger (rhizomes of *Zingiber officinale*) and the essential oil were tested using [^{14}C]guanidinium influx into N1E-115 cells and the isolated rat ileum in order to identify their activity in inhibiting 5-HT_3 receptor function. The hexane extract proved to be the most active and yielded upon bioassay-guided fractionation nine constituents: [6]-, [8]-, [10]-gingerols, [6]- and [8]-shogaols which were previously shown as active *in vivo* against cytotoxic drug-induced emesis; [4]-gingerol, [6]-gingerdiol, diacetyl-[6]-gingerdiol and [6]-dehydrogingerdione have not been previously tested for anti-emetic or 5-HT_3 receptor antagonistic effects. Even though the latter four compounds are only minor constituents, their identification contributed towards the characterisation of a structure-activity relationship of this class of compounds. The order of potency for the nine constituents in the N1E-115 cell system was [6]-gingerdiol ≈ diacetyl-[6]-gingerdiol ≈ [6]-dehydrogingerdione ≈ [6]-shogaol ≥ [8]-shogaol ≈ [8]-gingerol > [10]-gingerol ≥ [6]-gingerol > [4]-gingerol.

Key words
Zingiber officinale · Zingiberaceae · anti-emetic activity · 5-HT_3 receptor antagonists · N1E-115 cells · gingerol derivatives · SAR

Introduction

Crude ginger (rhizome of *Zingiber officinale* Roscoe) has been used since ancient times in traditional medicine as an anti-emetic. In recent years, *in vivo* experiments have been carried out to show its anti-emetic properties, particularly against acute cytotoxic drug-induced emesis [1], [2], [3]. The activity was at least in part attributed to the presence of the active principles [6]-, [8]-, [10]-gingerols, as well as [6]-, [8]- and [10]-shogaols [4], [5] (Fig. **1**). Several mechanisms of action have been proposed to explain the anti-emetic action without a clear outcome. Some authors have suggested that these active principles act through a central mechanism [4], while others implicated either an inhibitory effect on 5-HT_3 receptors in a way similar to the setrons or a free radical scavenging effect [1]. One *in vitro* trial showed that a ginger acetone extract as well as [6]-, [8]- and [10]-gingerols were able to inhibit serotonin-induced contractions of the isolated guinea-pig ileum and concluded that they all act by blocking 5-HT_3 receptors [6].

In the present paper, an attempt was made to isolate those active principles from ginger having a potential 5-HT_3 receptor blocking activity with the help of bio-guided fractionation using an *in vitro* cell assay based on the influx of [^{14}C]guanidinium into N1E-115 cells [7].

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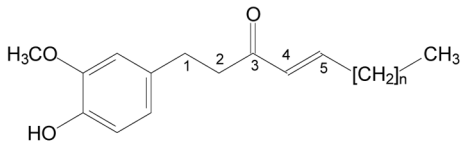
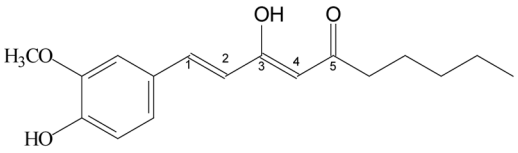
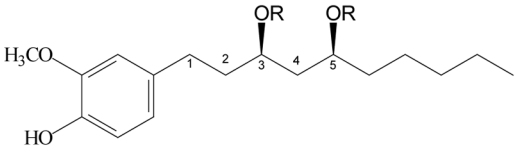


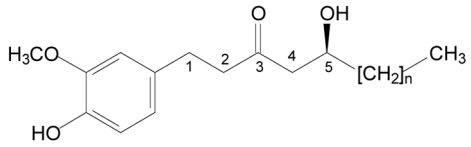
[6]-Shogaol: n = 4
[8]-Shogaol: n = 6



[6]-Gingerdiol: R = H
Diacetyl-[6]-gingerdiol: R = Ac



[6]-Dehydrogingerdione



[4]-Gingerol: n = 2
[6]-Gingerol: n = 4
[8]-Gingerol: n = 6
[10]-Gingerol: n = 8

Fig. **1** Compounds isolated from the hexane extract.

Materials and Methods

Extraction

Rhizoma Zingiberis officinalis, dried for 2 h at 100 – 105 °C, batch no. 01110836, was purchased from Finzelberg (Andernach). A voucher specimen has been deposited at the herbarium of the Institute for Pharmaceutical Biology and Phytochemistry, Münster (no. 187). The drug material was powdered and extracted successively with *n*-hexane (6 × 200 mL for 100 g of drug), EtOH (6 × 150 mL) and H_2O (3 × 200 mL) using an Ultra Turrax T50 [IKA, Staufen] (each time for 5 minutes at 6000 rpm). Extraction was done under cooling (ice bath) and light protection. All extracts were filtered, concentrated under reduced pressure at 25 °C, dried and kept at –20 °C. Each 100 g of drug yielded 4.2 g (= 4.5 mL) hexane extract, 2.3 g ethanolic fraction and 11.1 g aqueous fraction. In addition a steam distillate was prepared (distillation for 2 h yielded 0.85 mL essential oil for 100 g of drug).

Isolation and purification of compounds

The hexane extract was subjected to further fractionation using multilayer countercurrent chromatography (MLCCC): Ito multilayer coil separator-extractor, column capacity: 375 mL, 1.6 mm [Zinsser Analytik, Frankfurt FRG], 800 rpm, flow 1 mL/min, two-phase solvent system: *n*-heptane:EtOAc:MeOH:H_2O, 9 + 1 + 9 + 1, with the lower layer as stationary and the upper as the mobile phase. According to TLC analysis (SiO_2; toluene:EtOAc, 9 + 1, detection with anisaldehyde/sulphuric acid), the fractions with similar constituents were pooled to yield 5 fractions: HF1 (1 – 18 mL, 3 %), HF2 (19 – 42 mL, 29.4 %), HF3 (43 – 63 mL, 1.5 %), HF4 – 6 (64 – 946 mL, 24.4 %) and HF7 (stationary phase, 41.8 %).

The main active fraction HF7 was further purified by MLCCC using the same coil as described above (solvent system: *n*-heptane:EtOAc:MeOH:H_2O, 6 + 4 + 6 + 4, with the lower layer as stationary and the upper as mobile phase). According to TLC analysis, similar fractions were pooled, giving 5 sub-fractions, namely HF7F1 – 3 (1 – 231 mL), HF7F4 – 5 (232 – 406 mL), HF7F6 (407 – 515 mL), HF7F7 – 9 (516 – 843 mL), HF7F10 (stationary phase).

The sub-fractions were chromatographed on a preparative RP-18 column (Eurospher 100, 250 × 20 mm, 7 μm) [VDS Optilab, Montabaur, FRG] using a linear gradient of MeOH/H_2O from 70 : 30 to 100 : 0 over 40 min at a flow rate of 10 mL/min; detection at 280 nm. [6]-Gingerol was isolated from HF7F10 (t_R 10 min, yield 6.8 % of ginger hexane extract), [8]-gingerol from HF7F7 – 9 (16 min, 1.4 %) and [10]-gingerol from HF7F4 – 5 (21 min, 2.4 %). The $[\alpha]_D^{20}$ values of the isolates were + 11.4, + 11.2 and + 10.9, respectively (*c* 0.1, MeOH).

[4]-Gingerol and [6]-gingerdiol were isolated from HF7 over a small column (36 g silica gel) using flash chromatography. Elution was done with solvent steps with toluene/EtOAc in different ratios (100 + 0, 375 mL; 96 + 4, 375 mL; 94 + 6, 75 mL; 90 + 10, 100 mL; 80 + 20, 75 mL; 50 + 50, 50 mL; 0 + 10 050 mL) with fractions of 25 mL being collected. The column was then washed with 100 mL MeOH and 100 mL H_2O to give fraction 45. Fractions 1 – 37

(with traces of [6]-, [8]- and [10]-gingerols; TLC analysis) were pooled and applied to preparative TLC plates (pre-coated glass plates, silica gel 60 F_{254}, 20 × 20 cm, 0.5 mm, 12 mg/plate; toluene:EtOAc, 1 : 1; UV light 254 and 360 nm). The zones with Rf = 0.38 – 0.5 (below [6]-, [8]- and [10]-gingerols) were collected, eluted from the silica gel (MeOH) and evaporated to dryness. The residue was purified by means of preparative HPLC (Eurospher 100, RP-18, 250 × 20 mm, 7 μm, MeOH/H_2O, 60 + 40 for 30 min followed by a linear gradient of MeOH/H_2O, 60 + 40 to 100 + 0 over 40 min with a flow rate of 8 mL/min; t_R 16 min for [4]-gingerol and 41 min for [6]-gingerdiol, yields: 0.084% and 0.079% of ginger hexane extract, respectively); the compounds were finally freeze-dried; $[\alpha]_D^{20}$: 9.0 and 9.7, respectively (*c* 0.05, MeOH).

In a second isolation procedure, the hexane extract was fractionated again by MLCCC as described above using the same solvent system but a longer column (Ito multilayer coil separator-extractor, column: 450 mL capacity, 1.6 mm) and 950 rpm with a flow rate of 1.5 ml/min. the following fractions were obtained: HF1 – 3 (1 – 99 mL, 34%); HF4 – 6a (100 – 1288 mL, 22.2%); HF4 – 6b (1289 – 1477 mL, 1.8%); HF4 – 6/7 (1478 – 1603 mL, 1.2%); HF7a (1604 – 1828 mL, 1.1%); HF7b (1829 – 2161 mL, 2.1%); HF7c (2162 – 3241 mL, 6.5%); HF7d (stationary phase, 31.5%). Subfractions HF7b and HF7c were further purified by preparative HPLC (RP-18 column, 7 μm, 250 × 20 mm; 75 + 25 MeOH/H_2O, 8 mL/min for 40 min followed by a linear gradient of the same solvents from 75 + 25 to 100 + 0 over 20 min) to give [6]-shogaol (t_R 26 min) and diacetyl-[6]-gingerdiol (t_R 35 min); $[\alpha]_D^{20}$: 16.3 (*c* 0.05, MeOH), and [6]-dehydrogingerdione from HF7c (t_R 48 min). The compounds were finally freeze-dried.

[8]-Shogaol was identified in fraction HF4 – 6b by means of HPLC using authentic [8]-shogaol (gift of Dr. C. Duke, Sydney, Australia) as reference.

Structure elucidation

The structures of the isolates (Fig. **1**) were determined by their NMR data (^{13}C, ^1H, COSY, gHMBC, gHSQC, NOE), electro-spray ionisation mass spectroscopy (ESI-MS) in the positive or negative ion mode, UV spectra and $[\alpha]_D$ values in comparison with published data for the gingerols [8], [9], the shogaols [10], the gingerdiols [11], and [6]-dehydrogingerdione [12].

Preparation of solutions for pharmacological testing

All extracts and isolated compounds, except for the aqueous ginger extract were dissolved in DMSO and then diluted to the required concentration with the respective assay buffer. The final concentration of DMSO in any of the experiments was less than 0.3%. In this concentration, DMSO did not show any effect on the test systems. The aqueous ginger extract, as well as all other substances, were dissolved directly in the respective assay buffer.

N1E-115 cell culture

Mouse neuroblastoma cells of the clone N1E-115 [7] and of passage numbers 40 – 70 were grown in Dulbecco's modified Eagle's medium (DMEM) containing sodium pyruvate, glucose (1000 mg/L) and pyridoxine; the growth medium was supplemented with penicillin (100 IU/mL), streptomycin (100 μg/mL) and 10% foetal calf serum [Invitrogen, Karlsruhe FRG]. Cells were cultured in an incubator in a humidified atmosphere containing 5% CO_2 at 37 °C in cell culture flasks [Sarstedt, Nümbrecht FRG] and sub-cultured twice a week. Three days before starting [^{14}C]guanidinium influx experiments, cells were sub-cultured in 24-well cell culture clusters (2.5×10^4 cells in 1000 μL/well) [Biochrom, Berlin FRG] before being used.

5-HT-induced [^{14}C]guanidinium influx

After removal of the growth medium, cells were washed and pre-incubated for 20 min with 37 °C incubation buffer (300 μL/well) containing HEPES 25 mM, Tris 25, KCl 5.4, $MgSO_4 7 H_2O$ 0.98, D-glucose 5.5, choline chloride 135, bovine serum albumin 1 mg/mL and the drugs to be tested for inhibition of [^{14}C]guanidinium influx. Pre-incubation was carried out in a humidified atmosphere containing 5% CO_2 at 37 °C. After pre-incubation, the cells were incubated for a further 2.5 min with the same incubation buffer which in addition contained 5 μM [^{14}C]guanidinium chloride (specific activity 55 mCi/mmol) [Biotrend, Cologne FRG], the drug to be tested, and 100 μM serotonin creatinine sulphate monohydrate (5-HT) [Fluka, Seelze] at room temperature. Incubation was terminated by removing the incubation buffer and rapidly washing the cells twice with ice-cold washing buffer (HEPES 25 mM, Tris 25, KCl 5.4, $MgSO_4 7 H_2O$ 0.98, D-glucose 5.5, NaCl 135). Thereafter, the cells were dissolved in 0.5 mL 0.1% Triton X 100 and the [^{14}C]guanidinium content of the solution was determined by liquid scintillation counting. All experiments were carried out in duplicate or triplicate. 100 μM Tropisetron [Novartis Pharma, Frankfurt FRG] was used as a positive control.

Cpms of blanks were subtracted and the value obtained from serotonin stimulation (100 μM) normalised to 100% representing maximum [^{14}C]guanidinium influx. The counts from samples containing the drugs under investigation, after subtracting the blank count, were expressed as percentage of maximum [^{14}C]guanidinium influx.

Contraction studies using rat isolated ileum

Rats of either sex weighing 200 – 350 g were maintained on a 12 : 12 h light-dark cycle with free access to food and water before experiments (animal care was assured: A48/2003 Veterinaeramt Muenster). The animals were killed using ether and the ileum excised rapidly and flushed with 37 °C Krebs-Henseleit solution. Whole ileal segments, 2 – 3 cm in length, were suspended in a 10-mL organ bath under 1.0 g tension in carbogen-aerated Krebs-Henseleit solution ($NaHCO_3$ 25 mM, D-glucose 5.5, NaCl 119, KCl 4.7, $CaCl_2 2 H_2O$ 2.5, KH_2PO_4 1.2, $MgSO_4 7 H_2O$ 1.2), maintained at pH 7.4 and 37 °C. The ileal segments were allowed to equilibrate for 30 to 60 min until a straight base line was obtained. Initially a concentration-response curve with SR57227A (highly selective 5-HT_3 agonist) [Tocris, Bristol, UK] was made for each segment and a submaximal concentration evoking 50 – 75% of the maximal response was chosen (mostly 10 μM).

The drug to be tested was then added to the organ bath and left for a contact time of 60 sec, before repeating the chosen submaximal concentration of SR57227A. The ileum was washed several times after each drug addition until it returned to its previous base line. The washing time was at least 20 min to avoid effects produced by receptor desensitisation. Ondansetron

[GlaxoSmithKline, Munich, FRG] and granisetron [Bristol-Myers Squibb, Munich, FRG] were used as positive controls.

The effect of the different drugs was expressed as the ratio of the response to the chosen sub-maximal dose after and before drug addition as a percentage:

$$\frac{\text{response to agonist after addition of drug under investigation}}{\text{response to agonist without previous addition of drug under investigation}} \times 100$$

Results

All 3 ginger extracts as well as the ginger essential oil were tested on both the N1E-115 cell model and the rat isolated ileum. The hexane extract, the EtOH extract and the essential oil inhibited the 5-HT-induced [^{14}C]guanidinium influx into N1E-115 cells in a concentration-dependent manner (Fig. **2**) with an order of activity of hexane extract > EtOH extract > essential oil. The concentrations used were based on the yield of each extract (drug/extract ratio); the aqueous extract did not show any inhibitory effect on [^{14}C]guanidinium influx.

These results were confirmed on the isolated rat ileum (Fig. **3**). All three preparations inhibited the contractions of the ileum to SR57227A (highly selective 5-HT$_3$ agonist) in a concentration-dependent manner. Per se they had no apparent agonistic effect on the ileum. The aqueous extract, on the other hand, had an intrinsic agonistic effect on the ileum, which was particularly evident at higher concentrations. At lower concentrations, therefore, the aqueous extract apparently did not affect the responses of the ileum to SR57772A. However, because the highest concentration used of the aqueous extract (8 mg/mL) already pre-contracted the ileum to nearly twice the response to 10 μM SR57227A (Fig. **3**), responses of the latter could not be appropriately measured in the presence of such a strong pre-contraction and appeared subdued (Fig. **2**). The contractions evoked by the aqueous extract were not affected by pretreatment of the ileum with 100 μM atropine, 100 μM ondansetron or 320 μM tropisetron (data not shown), indicating that the contractions were not mediated through stimulation of muscarinic, 5-HT$_3$ or 5-HT$_4$ receptors.

Since the hexane extract was the most active in both models, it was fractionated by MLCCC and its fractions tested in the same assays. HF1 failed to show any effect in both models (Fig. **4**) for [^{14}C]guanidinium influx experiments). Both HF2 and HF3 had a weak inhibitory effect on the [^{14}C]guanidinium influx into N1E-115 cells, but while the effect of HF2 was concentration-dependent, that of HF3 seemed to be greater at the lower concentration than at the higher one (Fig. **4**). The two fractions, however, failed to affect the response of the isolated rat ileum to SR57227A (data not shown). Both HF4 – 6 and HF7 were equipotent in inhibiting the [^{14}C]guanidinium influx into N1E-115 cells in a concentration-dependent manner (Fig. **4**). A similar effect was exerted by the two fractions on the isolated rat ileum, where they both inhibited the contractions to SR57227A (Fig. **5**) in a concentration-dependent fashion. HF4 – 6 and HF7 shifted the SR57772A concentration-response curve to the right (insert to Fig. **5**), presumably in a non-competitive manner.

[6]-, [8]- and [10]-gingerols were the main constituents isolated from HF7. All 3 compounds inhibited the serotonin-induced [^{14}C]guanidinium influx into N1E-115 cells in a concentration-dependent manner in the following order of potency: [8]-gingerol > [10]-gingerol ≥ [6]-gingerol (Fig. **6**). Since the three com-

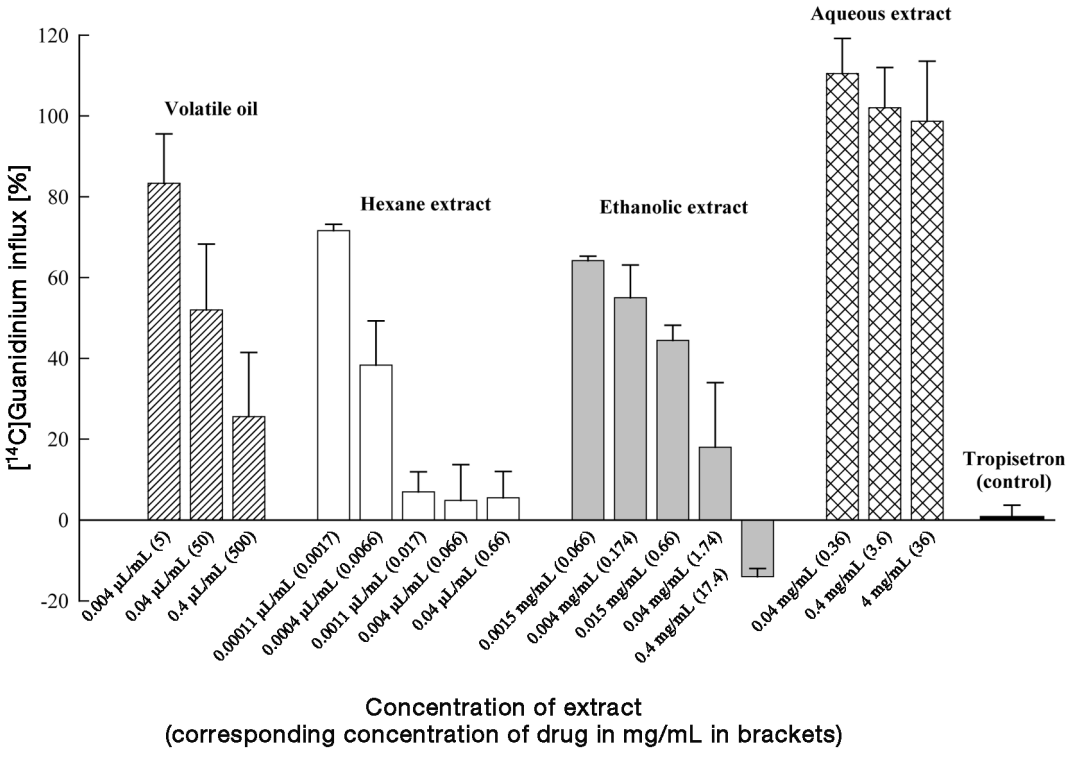


Fig. **2** Inhibition of 5-HT-induced [^{14}C]guanidinium influx into N1E-115 cells by various ginger extracts. Cells were incubated for 2.5 min at room temperature with 100 μM serotonin in the presence of different concentrations of essential oil, hexane extract, ethanolic extract, aqueous extract or 100 μM tropisetron (as control). Prior to stimulation with serotonin, cells were pre-incubated with the same concentration of extracts or tropisetron for 20 minutes. Bars represent mean values ± SEM from 3 – 5 experiments expressed as percentage of maximum [^{14}C]guanidinium influx.

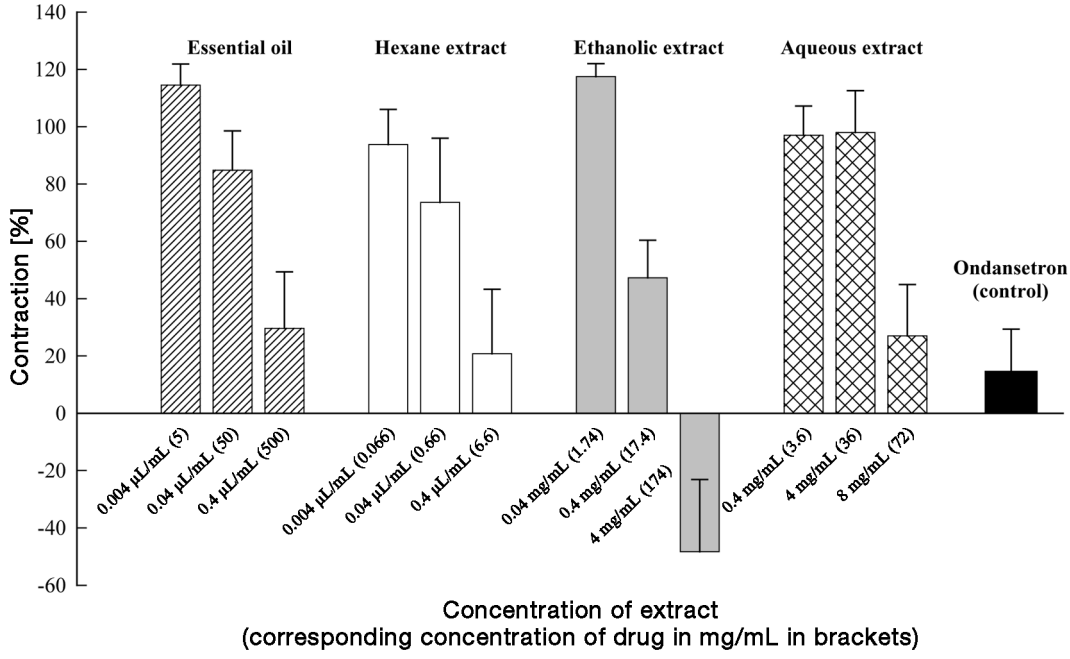


Fig. **3** Effect of different concentrations of various ginger extracts and on the isotonic contractions of the isolated rat ileum to SR57227A. Extracts or 100 μM ondansetron (as control) were left to act for 60 sec before measuring the response to SR57227A in the chosen sub-maximal dose (in most cases 10 μM). Bars represent mean values ± SEM from 3 – 6 experiments expressed as a percentage of the response to the chosen submaximal dose of SR57227A.

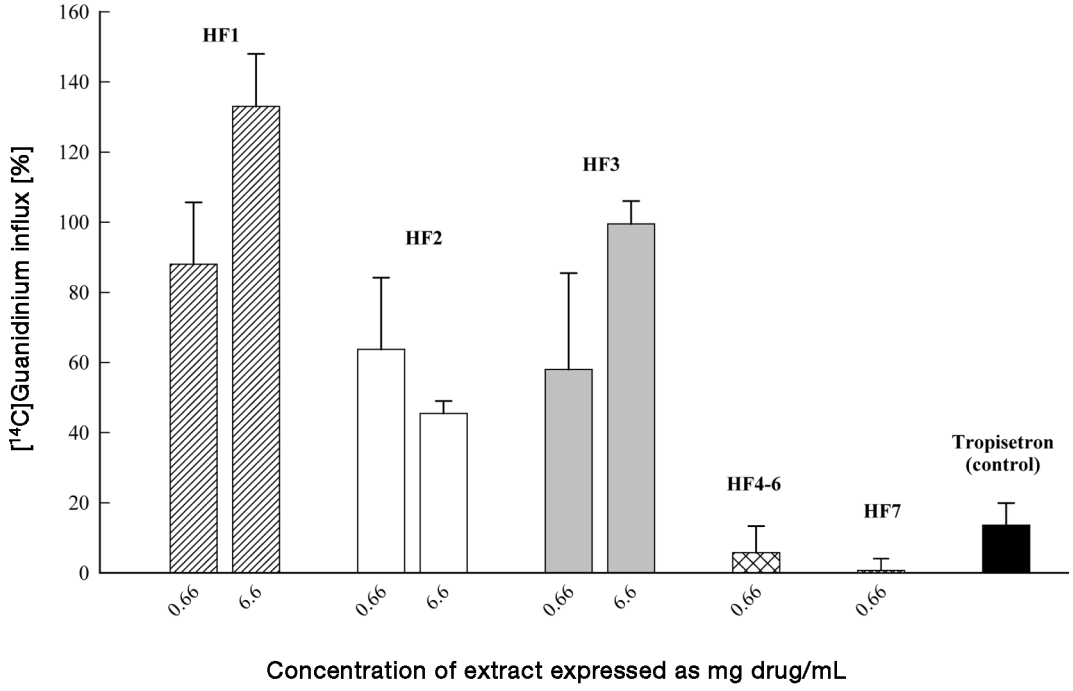


Fig. **4** Inhibition of 5-HT-induced [^{14}C]guanidinium influx into N1E-115 cells by various fractions of ginger hexane extract. Cells were incubated for 2.5 min at room temperature with 100 μM serotonin in the presence of different concentrations of hexane extract fractions 1 (HF1), 2 (HF2), 3 (HF3), 4 – 6 (HF4 – 6), 7 (HF7) or 100 μM tropisetron (as control). Prior to stimulation with serotonin, cells were preincubated with the same concentration of extract or tropisetron for 20 minutes. Bars represent mean values ± SEM from 3 – 4 experiments expressed as percentage of maximum [^{14}C]guanidinium influx.

pounds are present in the plant in different ratios ([6]-gingerol > [8]-gingerol > [10]-gingerol), they all seem to contribute to the overall activity of the ginger extract to a similar extent.

Five further compounds were isolated from HF7, namely [4]-gingerol, [6]-gingerdiol, diacetyl-[6]-gingerdiol, [6]-dehydrogingerdione and [6]-shogaol. All compounds inhibited the [^{14}C]guanidinium influx into N1E-115 cells in a concentration-dependent manner with [4]-gingerol being the least potent (Figs. **7** and **8**). Furthermore, a synthetic [8]-shogaol was tested using the same test system and showed good anti-serotoninergic activity (Fig. **8**). This compound was identified to be a main constituent of fraction HF4 – 6b.

Discussion

In recent years, different *in vivo* studies on various animal species have demonstrated the prophylactic effect of some ginger extracts and constituents against chemotherapy-induced emesis [1], [2], [4], [3], [5]. These results were substantiated by two preliminary clinical trials [13], [14], which showed the beneficial value of ginger against cytotoxic drug-induced nausea. In spite of these findings, only few attempts have been made to elucidate the exact mechanism by which ginger exerts its anti-emetic properties. Since 5-HT$_3$ receptors are known to play an important role in this type of emesis [15], one of the suggested mechanisms of action was through antagonism of 5-HT$_3$ receptors. This sug-

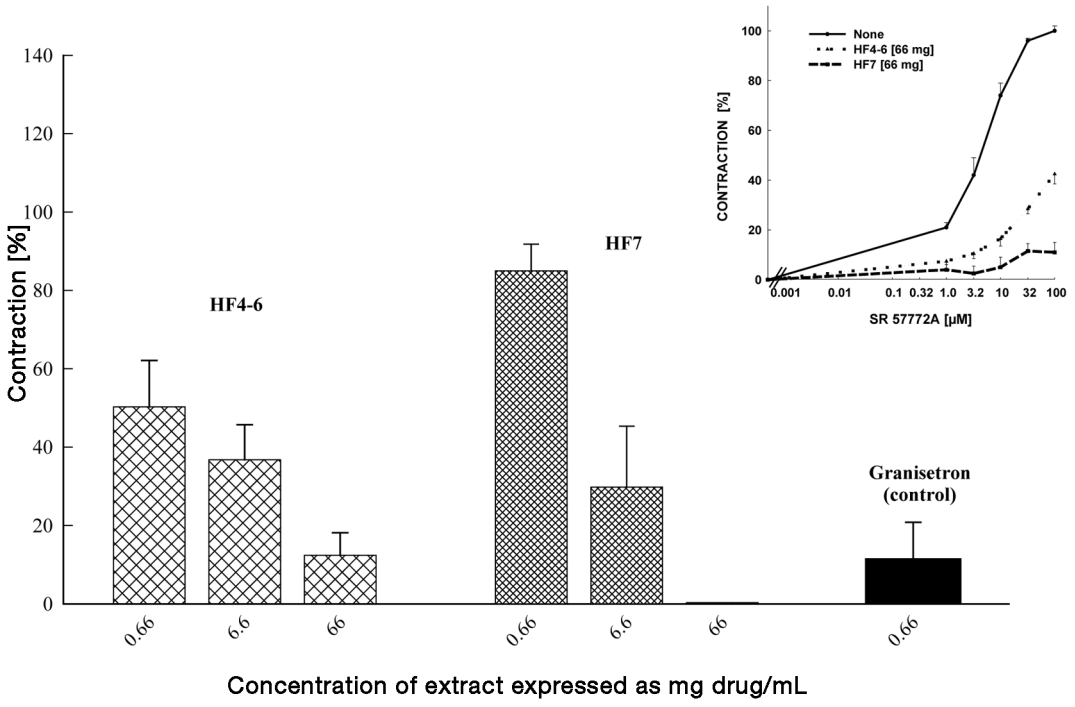


Fig. **5** Effect of different concentrations of the ginger extract fractions HF4 – 6 and HF7 on the isotonic contractions of the isolated rat ileum to SR57227A. Extract fractions or 100 μM granisetron (as control) were left to act for 60 sec before measuring the response to SR57227A in the chosen sub-maximal dose. Bars represent mean values ± SEM from 3 experiments expressed as a percentage of the response to 10 μM SR57227A. The insert shows the concentration response curves for rat ileum contraction induced by SR57227A in the absence and presence of 66 mg HF4 – 6 and HF7 (calculated from ginger drug). Mean ± SEM from 3 experiments expressed as percentage of the maximum effect.

gestion was supported by the *in vitro* studies of Yamahara et al. [6], and Huang et al. [16]. Yamahara et al. [6] showed that ginger acetone extract as well as [6]-, [8]- and [10]-gingerols inhibit the contractile response of the isolated guinea-pig ileum to serotonin. These authors, however, used in their experiments serotonin to contract the ileum and not a selective 5-HT_3 receptor agonist, and based their conclusions accordingly. The possible involvement of other 5-HT receptors present in the preparation [17], [18], however, cannot be ruled out. Furthermore, the gingerols tested were applied in only one concentration (with the exception of [8]-gingerol), which makes it difficult to draw any conclusive evidence from the experiments. Huang et al. [16] carried out a well designed experimental study on 3 different isolated organs containing serotoninergic receptors. However, these authors studied merely the 5-HT_3 receptor antagonistic effect of galanolactone, a diterpene lactone found only in Kintoki ginger (var. *rubens* Makino, present in Japan), but not in the officinal ginger [19]. The structure of galanolactone differs strongly from that of the gingerols and their analogues, and accordingly the results obtained do not allow for any definite conclusions to be drawn regarding the mode of action of other ginger components.

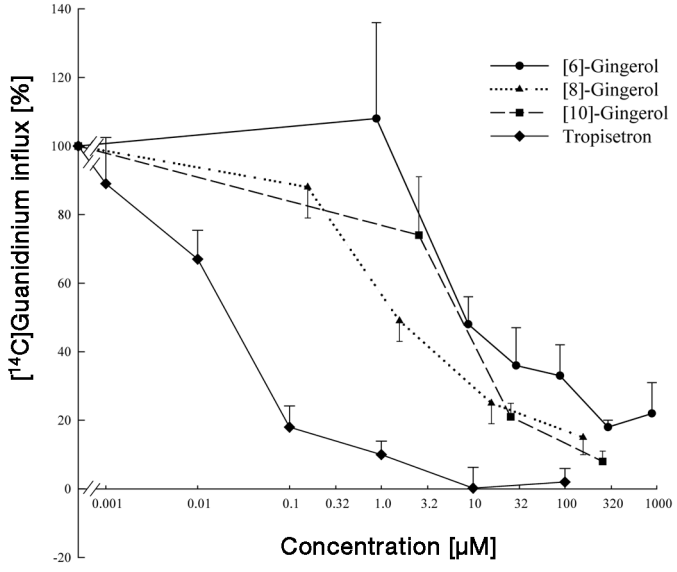


Fig. **6** Concentration response curves for the inhibitory effect of [6]-, [8]-, [10]-gingerols and tropisetron on 5-HT-induced [^{14}C]guanidinium influx into N1E-115 cells. Cells were incubated for 2.5 min at room temperature with 100 μM serotonin in the presence of different concentrations of [6]-, [8]-, [10]-gingerols or tropisetron (as control). Prior to stimulation with serotonin, cells were preincubated with the same concentration of gingerol or tropisetron for 20 minutes. Each point on the curves represents the mean value ± SEM from 3 – 7 experiments expressed as percentage of maximum [^{14}C]guanidinium influx.

Using N1E-115 cells, a cell line which has been used extensively to study the properties of 5-HT_3 receptors and the effects of different compounds on them [20], [21], we isolated in the present work nine active compounds from the ginger hexane extract. All nine compounds inhibited 5-HT_3 receptor function in a concentration-dependent manner. Five of them, namely [6]-, [8]-, [10]-gingerols, [6]- and [8]-shogaols, were previously shown to be effective against chemotherapy induced emesis [2], [4], [5]. The order of potency for these compounds on the functional test system examined in the present work ([6]-shogaol ≥ [8]-shogaol [8]-gingerol > [10]-gingerol ≥ [6]-gingerol; Fig. **1**) suggests that shogaols are at least equally potent to gingerols in inhibiting 5-HT_3 receptor function. These results are in agreement with previously published data [3], [4], [5], and thus attempts to stabilise the gingerols (to prevent their artificial conversion into shogaols) in a pharmaceutical preparation seem to be unnecessary.

With the use of the N1E-115 cell assay which, in contrast to the *in vivo* models used for bio-guided fractionation in earlier studies, only requires small quantities of test compounds, it was possible

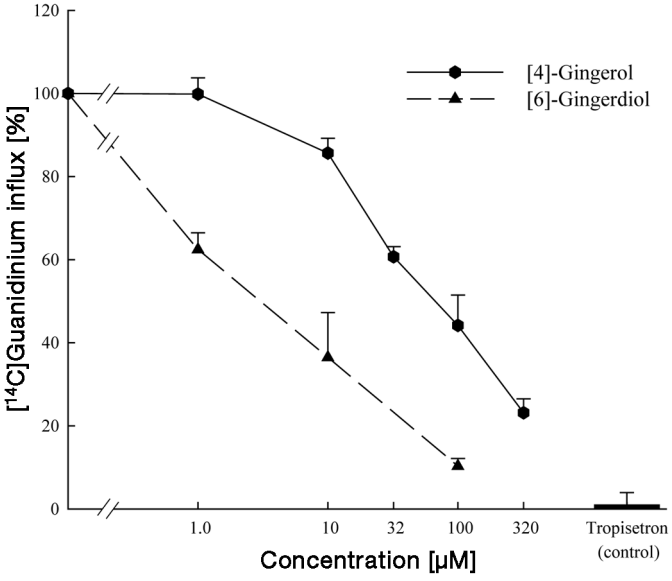


Fig. **7** Concentration-response curves for the inhibitory effect of 2 compounds isolated from the HF7 subfraction ZW3 on 5-HT-induced [^{14}C]guanidinium influx into N1E-115 cells. Cells were incubated for 2.5 min at room temperature with 100 μM serotonin in the presence of different concentrations of [6]-gingerdiol, [4]-gingerol or 100 μM tropisetron (as control). Prior to stimulation with serotonin, cells were preincubated with the same concentration of compound or tropisetron for 20 minutes. Each point on the curves represents the mean value ± SEM from 3 – 5 experiments expressed as percentage of maximum [^{14}C]guanidinium influx.

to isolate and test 4 minor ginger constituents ([4]-gingerol, [6]-gingerdiol, diacetyl-[6]-gingerdiol, [6]-dehydrogingerdione; Fig. **1**), which have never been tested before for their anti-emetic or anti-serotoninergic activity. The relative activity of the latter three was even higher when compared to gingerols and shogaols (Figs. **7** and **8**). Due to their presence in low concentrations in the drug, their contribution to the overall effect of a ginger extract is probably of minor significance, but their isolation and testing presents a further step in establishing a structure-activity relationship for the anti-emetic effect of gingerol analogues. This was recently attempted by Yang et al. [5] who examined the structure and anti-emetic activity of 19 natural and semi-synthetic diarylheptanoids and their analogues, including [6]-, [8]-, [10]-gingerols and [6]-, [8]-, [10]-shogaols in young chicks against copper sulphate-induced emesis. The authors classified the examined compounds into 2 groups: group A with 'A type' functional structure (e.g., shogaols) and group B with 'B type' functional structure (e.g., gingerols) (Fig. **1**). Apparently, active compounds must contain at least one aryl group bound to an alkyl side chain, which carries 2 electron-donating groups. The latter may either take the form of an oxygen functional group and a double bond (A type) or 2 oxygen functional groups (B type); in case of compounds having aryls on both sides, these should not be connected by a conjugated system of double bonds as realised in the curcuminoids (in the N1E-115 cells assay curcumin did not show any activity; unpublished results). It also seems that the electron-donating groups have to be connected to the aryl group by a two-carbon ethyl bridge to keep needed for activity [5].

Furthermore, the present findings indicate in addition to [5] that acetylation of the OH groups in the B type functional structure does not affect the activity of the compounds since [6]-gingerdiol and its diacetyl derivative exerted similar inhibitory effects on the serotonin-induced [^{14}C]guanidinium influx into N1E-115 cells. [6]-Dehydro-gingerdione (enol form in solution) interestingly was significantly active in the N1E-115 cells assay indicating that the "B type" as described by [5] has to be structurally extended in that the two-carbon bridge can also be an ethylene group.

The finding that [8]-gingerol is the most potent among the isolated gingerols, which is in agreement with the *in vitro* study published by Yamahara et al. [6], indicates that aliphatic side chains with in total 12 carbon atoms give structures with highest possible activity for the gingerols (possibly for all B type functional structures). However this does not seem to be true for the shogaols (A type), since [6]-shogaol was more potent than [8]-shogaol (Fig. **8**).

Besides the nine gingerols or gingerol analogues investigated in this study other fractions of the hexane extract, namely HF4 – 6, as well as the essential oil obtained from ginger also exerted good anti-serotoninergic effects. The presence of other classes of compounds acting with similar effects on 5-HT$_3$ receptors is currently under investigation.

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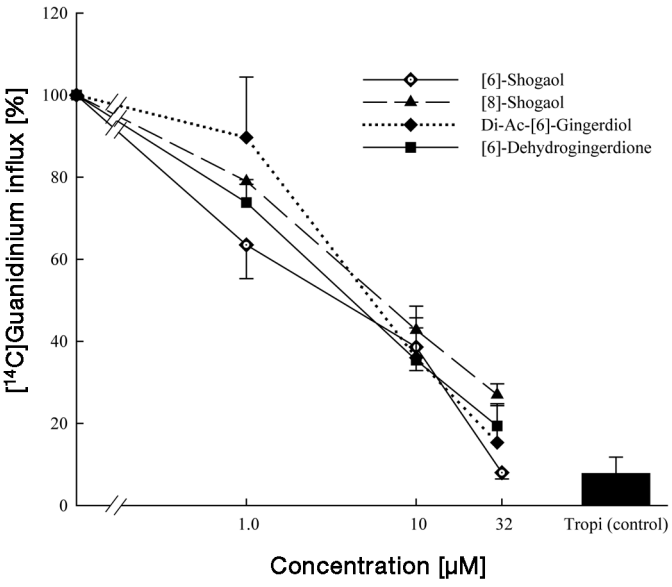

Fig. **8** Concentration response curves for the inhibitory effect of various compounds isolated from ginger hexane extract on 5-HT-induced [^{14}C]guanidinium influx into N1E-115 cells. Cells were incubated for 2.5 min at room temperature with 100 μM serotonin in the presence of different concentrations of [6]-, [8]-shogaols, diacetyl-[6]-gingerdiol, [6]-dehydrogingerdione or 100 μM tropisetron (as control). Prior to stimulation with serotonin, cells were preincubated with the same concentration of compound or tropisetron for 20 minutes. Each point on the curves represents the mean value ± SEM from 3 – 7 experiments expressed as percentage of maximum [^{14}C]guanidinium influx.

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