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In silico investigation into the interactions between murine 5-HT_3 receptor and the principle active compounds of ginger (*Zingiber officinale*)

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ABSTRACT

Gingerols and shogaols are the primary non-volatile actives within ginger (*Zingiber officinale*). These compounds have demonstrated *in vitro* to exert 5-HT_3 receptor antagonism which could benefit chemotherapy-induced nausea and vomiting (CINV). The site and mechanism of action by which these compounds interact with the 5-HT_3 receptor is not fully understood although research indicates they may bind to a currently unidentified allosteric binding site. Using *in silico* techniques, such as molecular docking and GRID analysis, we have characterized the recently available murine 5-HT_3 receptor by identifying sites of strong interaction with particular functional groups at both the orthogonal (serotonin) site and a proposed allosteric binding site situated at the interface between the transmembrane region and the extracellular domain. These were assessed concurrently with the top-scoring poses of the docked ligands and included key active gingerols, shogaols and dehydroshogaols as well as competitive antagonists (e.g. setron class of pharmacologically active drugs), serotonin and its structural analogues, curcumin and capsaicin, non-competitive antagonists and decoys. Unexpectedly, we found that the ginger compounds and their structural analogs generally outscored other ligands at both sites. Our results correlated well with previous site-directed mutagenesis studies in identifying key binding site residues. We have identified new residues important for binding the ginger compounds. Overall, the results suggest that the ginger compounds and their structural analogues possess a high binding affinity to both sites. Notwithstanding the limitations of such theoretical analyses, these results suggest that the ginger compounds could act both competitively or non-competitively as has been shown for palonosetron and other modulators of CYS loop receptors.

1. Introduction

Chemotherapy-induced nausea and vomiting (CINV) poses a major obstacle to patients often resulting in treatment cessation due to its severity and intolerability. Without appropriate antiemetic prophylaxis, up to ninety percent of all cancer patients receiving chemotherapy may experience nausea and/or vomiting [1]. In a recent review of the incidence of CINV, around twenty to forty percent of patients failed to respond to the current antiemetic treatments in relation to either vomiting or nausea with nausea being less well managed [2]. Nausea and delayed CINV are reported as particular challenges in clinical practice. Thus a significant impetus exists to develop more effective treatments.

This study focuses on one of the primary pathways of emesis relating to CINV – the stimulation of vagal afferent nerves due to high levels of serotonin released from the mucosal enterochromaffin cells of the gut [1,3,4]. Serotonin allosterically activates the 5-HT Type 3 (or 5-HT_3) ion channel by binding to a site distinct from the transmembrane region where channel opening occurs, facilitating neuronal depolarisation [5].

The cationic 5-HT_3 receptor belongs to the CYS loop superfamily of ligand-gated ion channels (LGICs) along with nicotinic acetylcholine receptors (nAchR) and anionic g-aminobutyric acid receptor ($GABA_A$ and $GABA_C$) and glycine receptor [6]. The function of 5-HT_3 receptors is intricately fine-tuned by the binding of other molecules and ions in and adjacent to the channel, either extracellularly or within the membrane region. For example, all CYS-loop receptors are allosterically regulated by zinc ions binding at multiple locations [7]. Both anions and cations can enter the pore where ion filtering is controlled by specific residues lining

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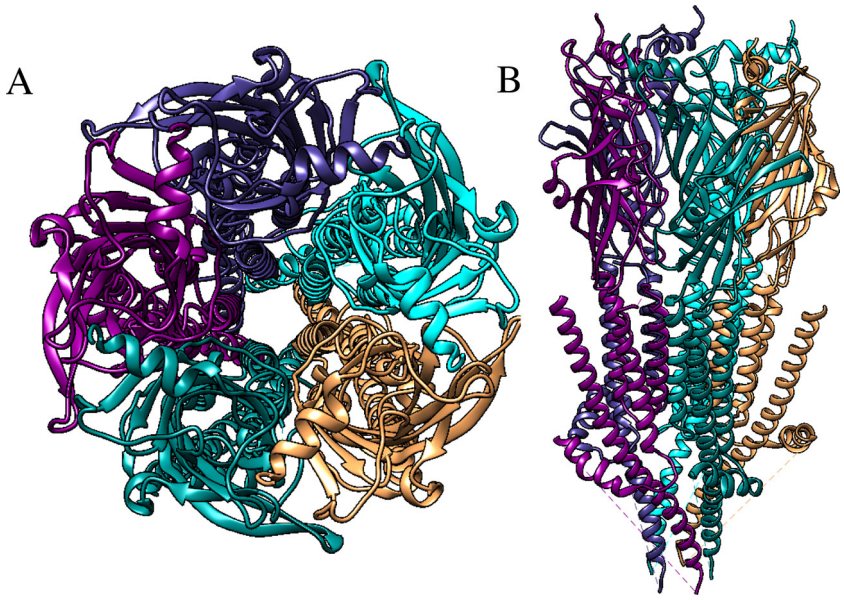


Fig. 1. Pentameric subunit arrangement of the 5-HT_3 receptor extract from PDB entry 4pir. (A): Top view (B): side view ECD containing (top), alpha helical TM domain and intracellular domain.

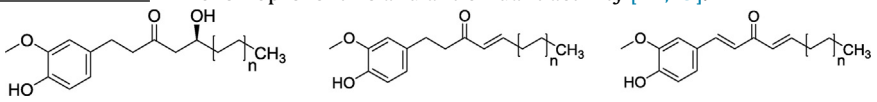
the narrow region of the pore at the cytoplasmic end of the transmembrane domain (TMD) extending into the TMI-II cytoplasmic loop [8]. A second ion filter controlling ion flow has been observed in the 5-HT_3 receptor at the intracellular transmembrane TMIII-IV loop [9].

CYS loop receptors share significant structural similarity consisting of a pentameric assembly of subunits with three domains: N-terminal extracellular domain (ECD) with the 15 amino acid CYS-loop disulphide; transmembrane domain with four helices, MI-IV and intracellular domain (ICD) consisting of a long loop between MIII and MIV (Fig. 1). The C-terminus is extracellular.

Five distinct subunits (A to E) have been identified for the 5-HT_3 receptor whereby A, B, C & E are similar while subunit 5-HT_{3D} lacks an amino terminal CYS loop [10]. The subunits in the functioning unit are either arranged homo or heteromerically around a cation-specific, water filled central pore. Only the A subunit has been shown to form functional homomeric receptors and, importantly, the presence of the A subunit was required in all receptors. Adding to the functional complexity, heteromeric receptors contain more possible sites for allosteric modulation than homomeric receptors [11]. Davies et al. [11] described a kinetic model of the 5-HT_3 receptor function delineating between open, closed and desensitized states.

A number of agonists and antagonists have been identified which are able to displace serotonin [12]. Among those that have significantly improved control of CINV are the "setron" class of antiemetics. Ondansetron and granisetron as well as the more recently introduced palonosetron, for example, are important tools not only for CINV but also emesis related to anaesthesia, surgery and radiotherapy [13–16]. Recently observations of multiple modes of inhibition by palonosetron, for example, exhibiting pseudo irreversible inhibition at the serotonin site but also acting at a distinct allosteric site, exemplifies the complexity of modulation and challenges to medicinal chemists [17–21]. This phenomenon has also been established for other CYS loop receptors.

Empirical evidence from *in vitro* and clinical data suggests ginger (*Zingiber officinale*) may be an effective treatment against several types of nausea including morning sickness, motion sickness and chemotherapy-induced nausea and vomiting [22,23]. Gingerols (for example **6G-10G**) constitute the principle, non-volatile, pungent components of ginger and have been associated with pharmacological effects including anti-inflammatory, anti-pyretic, angiogenesis, chemopreventive and antioxidant activity [24,25].



These phenols contain an unbranched alkyl chain differing in length. Minor components include the more oxidized shogaols (for example, **6S-10S**) or dehydroshogaols (for example, **6DHSG-10DHSG**). In contrast to the gingerols, both shogaols and dehydroshogaols contain an α,β-unsaturated carbonyl group (Michael acceptor moiety) known to possess antioxidative activity and chemoprotective effects [26]. The principle compounds in ginger, gingerols and shogaols, have been demonstrated to inhibit serotonin-mediated signaling and that this interaction could be mediated through a currently unidentified binding site [27–29]. *In vitro* studies by Abdel Aziz found that **6S**, **6G, 8G** and **10G** inhibited 5-HT_3-induced contractions of the isolated guinea-pig ileum. Since these same compounds were unable to displace the competitive antagonist, [^{3}H]GR65630, from the serotonin binding site, a non-competitive mechanism was proposed. These results were corroborated by an *in vitro* study by Walstab et al. [10] which indicated that ginger was able to inhibit the activation of human 5-HT_3 receptors and that this was likely via non-competitive mechanisms. Additionally, since pre-incubation with **6G** produced increased inhibition it was proposed that its binding site may be relatively inaccessible such as that of the transmembrane channel. As Walstab et al. noted, when combined with standard 5-HT_3 antagonists, the non-competitive binding of ginger compounds could potentially provide an additive effect to the control of nausea and vomiting in clinical practice. Indeed, clinical trials have reported a significant improvement in CINV where ginger was combined with standard treatment with a setron class drug [22,30].

Recently, the crystal structure of the murine 5-HT_3 receptor in the apo (or unbound) form was solved using X-ray crystallography