

## 5-HT<sub>3</sub> Receptors

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**Abstract:** The 5-HT<sub>3</sub> receptor is a member of the Cys-loop family of ligand-gated ion channels. These receptors are located in both the peripheral and central nervous systems, where functional receptors are constructed from five subunits. These subunits may be the same (homopentameric 5-HT<sub>3A</sub> receptors) or different (heteropentameric receptors, usually comprising of 5-HT<sub>3A</sub> and 5-HT<sub>3B</sub> receptor subunits), with the latter having a number of distinct properties. The 5-HT<sub>3</sub> receptor binding site is comprised of six loops from two adjacent subunits, and critical ligand binding amino acids in these loops have been largely identified. There are a range of selective agonists and antagonists for these receptors and the pharmacophore is reasonably well understood. There are also a wide range of compounds that can modulate receptor activity. Studies have suggested many diverse potential disease targets that might be amenable to alleviation by 5-HT<sub>3</sub> receptor selective compounds but to date only two applications have been fully realised in the clinic: the treatment of emesis and irritable-bowel syndrome.

**Key Words:** Serotonin receptor, ligand-gated ion channel, 5-HT<sub>3</sub> receptor, ligand binding, antagonist, agonist, pharmacology, disease.

### INTRODUCTION

5-HT<sub>3</sub> receptors are ligand-gated ion channels (LGIC) and therefore differ from all other 5-HT (serotonin) receptors whose actions are mediated *via* G proteins. Their structure and function has placed them in the Cys-loop family of ligand-gated ion channels. Similar to other members of this family, the 5-HT<sub>3</sub> receptor is composed of five symmetrically arranged subunits that surround a central ion-conducting pore (Fig. (1)). The 5-HT<sub>3A</sub> receptor subunit is able to form functional homomeric receptors, and also combines with the more recently cloned 5-HT<sub>3B</sub> subunit to create hetero-pentamers that display distinct electrical and pharmacological properties [1, 2]. Genes for 5-HT<sub>3C</sub>, 5-HT<sub>3D</sub> and 5-HT<sub>3E</sub> subunits have also been described, but to date these subunits have not been characterised [3].

Experiments on functional chimaeric receptors that combine regions from different members of the LGIC family has developed the idea of these receptors as modular structures that contain functionally distinct extracellular and transmembrane domains [4, 5]. The extracellular domain is responsible for ligand binding and is therefore the site of action of agonists and competitive antagonists. The transmembrane domain, which in this simplified definition includes the intracellular loop, controls the movement of ions across the cell membrane and is primarily responsible for electrical properties such as ion selectivity, channel rectification and conductance [1, 6-9].

There are currently no high resolution structures of the 5-HT<sub>3</sub> receptor, but the availability of crystal structures for the closely related acetylcholine binding protein (AChBP) has substantially enhanced our understanding of the extracellular

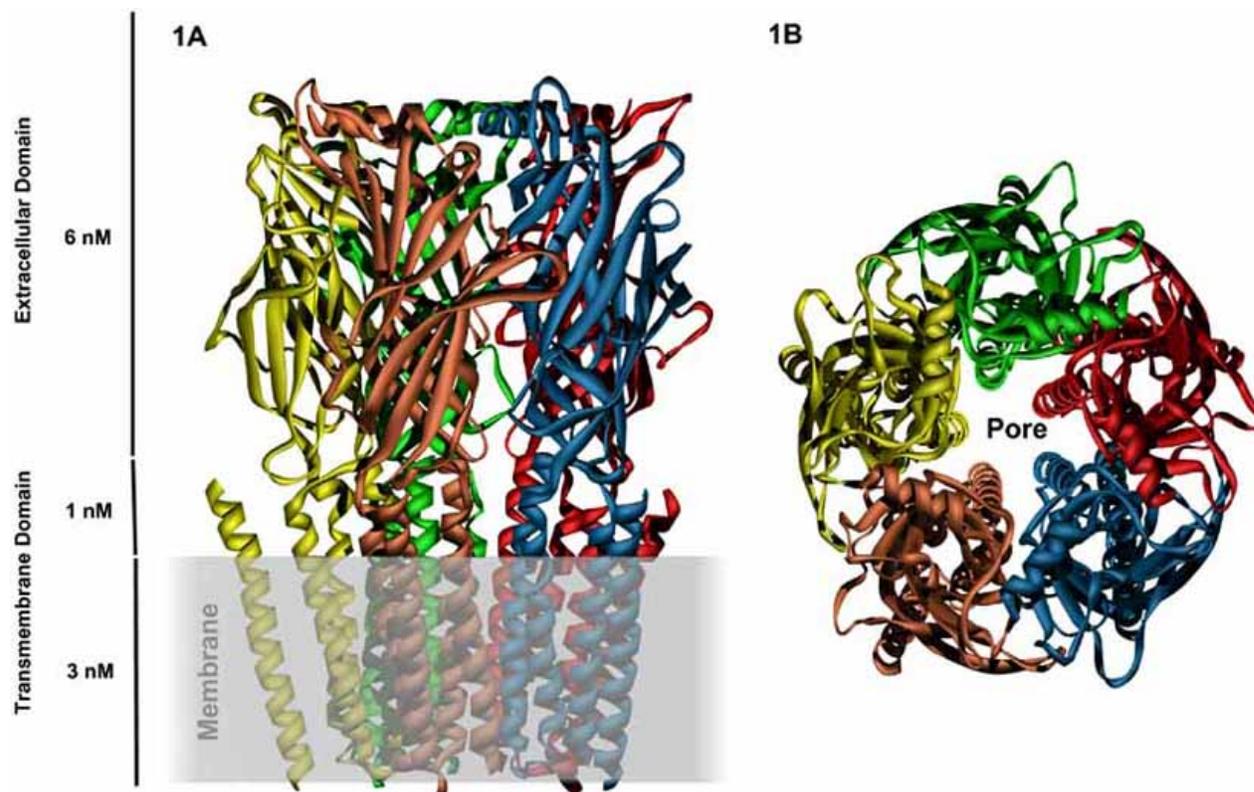
domain [10-12]. Using these structures, and results from mutagenesis studies, an image of the 5-HT<sub>3</sub> receptor ligand-binding site has emerged [13-19]. Here we will review these studies, in addition to providing information on the distribution, function and pharmacology of these receptors. In particular we will show how structure-function studies have provided us with a detailed image of the 5-HT<sub>3</sub> pharmacophore, and will discuss the current and future therapeutic potential of compounds that act *via* this receptor.

### DISTRIBUTION OF 5-HT<sub>3</sub> RECEPTORS

5-HT<sub>3</sub> receptors are located in both the peripheral (PNS) and central (CNS) nervous systems. Activation of these receptors in the PNS suggest they play a role in a variety of sympathetic, parasympathetic and sensory functions [20-22]. As might be expected due to their role in emesis, 5-HT<sub>3</sub> receptors are involved in information transfer in the gastrointestinal tract, and in the enteric nervous system they regulate gut motility and peristalsis [23]. They also play an important role in the urinary tract, and indeed expression of hypersensitive and constitutively active 5-HT<sub>3</sub> receptors in mice lead to excitotoxic neuronal cell death, resulting in early death due to uropathy [24].

5-HT<sub>3</sub> receptors in the CNS may play roles in a variety of functions including emesis, cognition and anxiety. They are located in many brain areas including cortex, hippocampus, nucleus accumbens, substantia nigra, and ventral tegmental area, with highest levels in the brain stem, especially areas involved in the vomiting reflex such as the area postrema and nucleus tractus solitarius, [22, 25, 26]. 5-HT<sub>3</sub> receptor activation enhances the release of a variety of neurotransmitters, including dopamine, cholecystokinin and GABA, and 5-HT<sub>3</sub> receptors have been localised to both pre and post-synaptic nerve terminals [27, 28]. There may be differential cellular localization of pre- and/or postsynaptic 5-HT<sub>3</sub> receptors within different central regions, depending on the

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**Fig. (1).** A homology model of the extracellular and transmembrane domains of the 5-HT<sub>3</sub> receptor. In Fig. (1A), the receptor is shown from the side and the position of the membrane is highlighted as a grey box. In Fig. (1B) the receptor is shown from above, looking down towards the membrane and through the central ion-conducting pore. The structure was created from a fusion of homology models based upon the crystal structure of AChBP (PDB ID; 1i9b) and cryo-electron microscopy of the nAChR (PDB ID; 1oed).

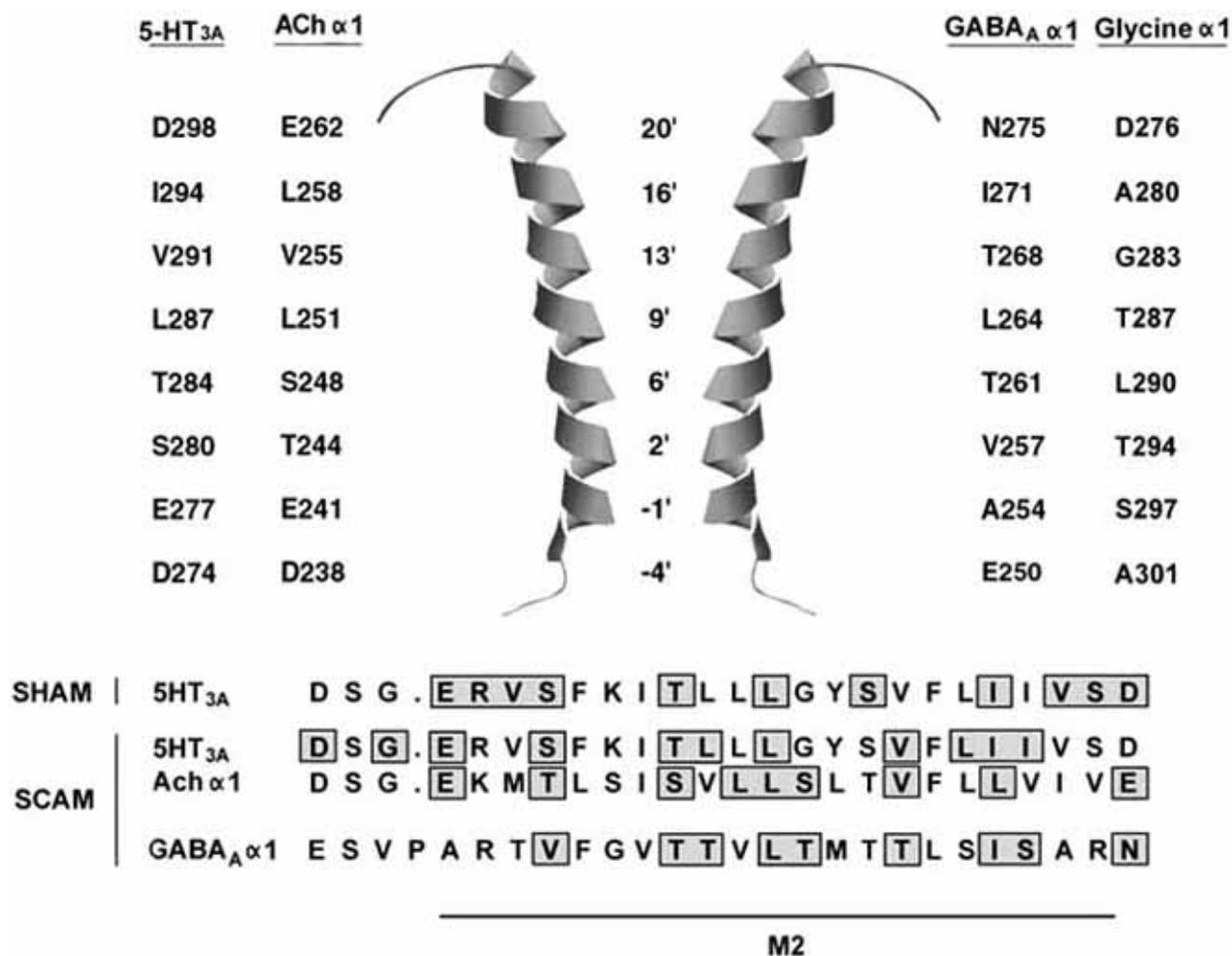
nature of the neurons containing 5-HT<sub>3</sub> receptors. For instance, 5-HT<sub>3</sub> receptor immunoreactivity was most abundant in postsynaptic dendrite sites in the hippocampus, but was primarily associated with presynaptic nerve endings in amygdale [25, 29].

5-HT<sub>3</sub> receptors in the PNS and CNS may be constructed of different subunits. All receptors contain an A subunit, but the distribution of B subunits is not yet clear. Immunochemical studies suggest that 5-HT<sub>3B</sub> receptor subunits are restricted to the PNS [21, 30], but in situ studies have shown 5-HT<sub>3B</sub> receptor subunit mRNA is present in human brain [1]. Other immunocytochemical studies have reported the presence of B subunits in rat hippocampal neurons [31], and thus it may be that they exist in the CNS in low abundance or in highly discretely localised cell populations. Interestingly, the reports suggest that the 5-HT<sub>3B</sub> receptor subunit is expressed in anatomical structures that are involved in drug induced emesis, although there has been no direct link between heteromeric receptor assemblies and the effects of antiemetic drugs. There is also some evidence that 5-HT<sub>3</sub> receptor subunits may co-express with subunits from other ligand-gated ion channels such as the nACh 4 subunit [32]. Receptors constructed from different subunits have implications for drug therapy, as discussed later.

### 5-HT<sub>3</sub> RECEPTOR FUNCTION

Homomeric and heteromeric 5-HT<sub>3</sub> receptors mediate a rapidly activating, desensitizing, inward current, which is predominantly carried by sodium and potassium ions [33, 34]. Ion substitution experiments have also established that the homomeric channel is permeable to calcium ions and small organic cations [35], although when the 5-HT<sub>3A</sub> receptor subunit is co-expressed with the 5-HT<sub>3B</sub> receptor subunit most of this permeability is lost [36].

The channel is formed from the second transmembrane segment (M2), and is predominantly  $\alpha$ -helical [37, 38]. Studies in the 5-HT<sub>3</sub> receptor have shown that neutralisation of a ring of negative charges at -1' (E-1'A) results in a receptor that is non-selective and adding a ring of positive charge to this receptor at the 19' (S19'R) position changes it to anion selective (Fig. (2)) [39]. This shows that charged residues close to M2 play a role in ion selectivity, although it is possible that the intracellular M3-M4 region of the 5-HT<sub>3</sub> receptor is also important in concentrating the appropriately charged species, as suggested for the nACh receptor [9, 40]. These data suggest that the loss of the glutamate residue at the -1' position in the 5-HT<sub>3B</sub> receptor subunit may also have implications for ion selectivity.



**Fig. (2).** M2 channel lining residues from four members of the ligand-gated ion-channel family. Supposed pore lining residues are shown next to the M2  $\alpha$ -helix (PDB ID; 1oed). Members of the family that have been studied using SCAM and SHAM are shown in the alignment below and amino acids that have been identified as accessible to modifying sulfydryl reagents or transition metal cations are highlighted as boxes in the 5-HT<sub>3</sub> [37, 149], ACh [150] and GABA<sub>A</sub> [151] receptors. Rings of charged amino acids are located at positions -4', -1' and 20'. Accession numbers for the alignment are: 5-HT<sub>3A</sub> Q605711, nACh 1 P02710, GABA<sub>A</sub> 1 P14867, Glycine 1 P23415.

### LIGAND BINDING SITE OF THE 5-HT<sub>3</sub> RECEPTOR

There is currently no high resolution structural data for the 5-HT<sub>3</sub> receptor. However, the extracellular N-terminal domain is homologous to AChBP, whose structure has been resolved to 2.1Å [11]. These data have allowed homology models of the 5-HT<sub>3</sub> receptor to be constructed, which show that the binding site for agonist and antagonists lies between the faces of two adjacent subunits and is formed by the convergence of three 'loops' (A-C) from the 'principal' subunit and three 'loops' (D-F) from the adjacent or 'complementary' subunit (Fig. 3) [16, 41]. Key residues in these loops in the homomeric 5-HT<sub>3A</sub> receptor are described in more detail below.

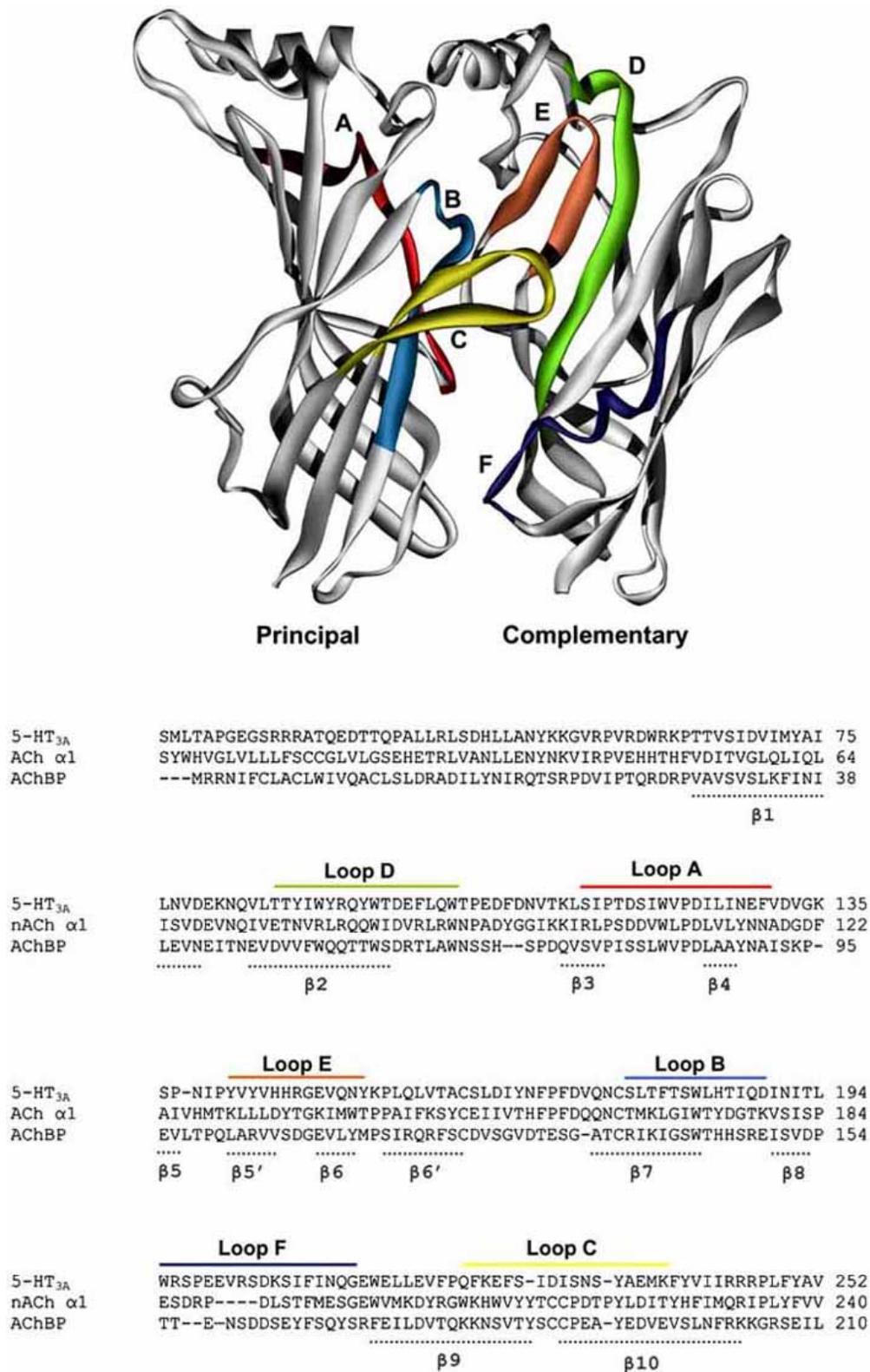
#### Loop A

Only residue N128 from loop A has been proposed to be in the binding pocket [41, 42]. This residue is equivalent to

Y93 of the nAChR  $\gamma$  subunit and Y89 of AChBP, which have both been identified as critical residues in ligand binding [10, 43]. However, data suggest that this residue is not a critical binding residue in the 5-HT<sub>3</sub> receptor as N128 mutations do not significantly alter the binding efficiency of antagonists [16]. In contrast the adjacent residues E129 and F130 modify both binding and function, and changing F130 to asparagine even allows the receptor to be activated by acetylcholine, indicating this may be the most critical loop A residue [17, 18]. It is also possible that this region is involved in receptor assembly, as mutation of W121 and P123 results in receptors that no longer reach the cell surface [44, 45].

#### Loop B

The most important B loop residue is W183, which is critical for both ligand binding and function. It has been



**Fig. (3).** Adjacent subunits (principal and complementary) showing the positions of the main binding loops within the extracellular domain. Only two of the five subunits have been shown for ease of viewing. An alignment of the 5-HT<sub>3A</sub>, nAChR  $\alpha 1$  and AChBP sequences is shown below. The binding loops of the receptors are indicated by lines above the alignment and their location can be seen in the structure above. The positions of the  $\beta$ -sheets are shown by grey lines beneath the text and are taken from the AChBP protein crystal structure [10]. Accession numbers for the 5-HT<sub>3A</sub>R, nACh  $\alpha 1$  and AChBP protein sequences are Q6J1J7, P02710 and P58154 respectively.

extensively investigated using both natural and unnatural amino acid mutagenesis and these studies have revealed that W183 forms a cation- bond with the primary amine of 5-HT [15, 46]. This residue is equivalent to W149 in the nACh 1 subunit, which also forms a cation- interaction with acetylcholine [47]. The equivalent tryptophan residue in AChBP (W143) is a key component of both nicotine and carbamylcholine binding, and equivalent aromatic residues in GABA<sub>A</sub> and glycine receptors have also been shown to play critical roles in ligand binding and function [43, 48-50].

### Loop C

A number of studies have been performed on loop C residues, which differ more between species than in any other loop of the 5-HT<sub>3</sub> receptor. The most important aromatic residue is probably Y234. Y234 is involved in ligand binding whilst mutation of the closely positioned Y240 does not have any significant effects [14]. Unnatural amino-acid mutagenesis has revealed that an aromatic residue at position 234 is essential for both binding and function, while removal of the hydroxyl group produced receptors that had unaltered ligand binding, but an increased EC<sub>50</sub> [13]. Other studies have attempted to find the residues responsible for the differing pharmacology of rodent and human 5-HT<sub>3</sub> receptors, but point mutations throughout the loop C region did not identify any residues that were essential for binding of the agonist *m*-chlorophenylbiguanide (*m*CPBG) or the antagonist *d*-tubocurarine (*d*-TC), suggesting multiple regions of the binding site are important [51, 52].

### Loop D

A number of residues in loop D have been shown to be important for agonist and antagonist interactions. Aromatic residues are required at positions W90 and W95. The former is critical for ligand binding, whilst the latter affects cell surface expression [16, 46]. Aromatic contacts have also been demonstrated in AChBP between the residue that is equivalent to W90 (W53) and the agonist nicotine [11, 53]. The equivalent residues in nACh ( W55, W57, W55) and GABA<sub>A</sub> ( F64) receptors are also important in binding, indicating that this position is functionally similar among many members of the LGIC family [11, 54, 55]. Other residues that may play a role in the binding site of the 5-HT<sub>3</sub> receptor are Y91, R92 and Y94 [14, 19].

### Loop E

Sequence variability in this loop can be seen both between subunits of the same species and the same subunit in different species, suggesting that the structure in this region may differ according to the stoichiometry of the receptor and/or the species. Scanning alanine mutagenesis of loop E has revealed that Y143, G148, E149, V150, Q151, N152, Y153 and K154 may be important for granisetron binding, and indeed mutation of G148 and V150 completely abolished binding [56]. The two tyrosine residues Y143 and Y153 have been further studied using unnatural amino acid mutagenesis which has shown that they both play roles in function, and Y153 also has a role in ligand binding [13, 14].

### Loop F

The role of loop F residues have yet to be elucidated. In the AChBP crystal structure, the loop F region was poorly resolved [10] and its current location on the homology model on the 5-HT<sub>3</sub> receptor is only tentative. To date the only mutational information is from studies which implicated W195 and S206 as potentially important residues [16,46].

## PHARMACOLOGY

### 5-HT<sub>3</sub> Receptor Agonists

There are a range of 5-HT<sub>3</sub> selective agonists, which show different efficacies depending on species and receptor stoichiometry. The first selective agonists were 2-Methyl-5-HT (2-Me-5-HT), phenylbiguanide (PBG) and *m*CPBG [57-60]. 2-Me-5-HT is less potent than 5-HT and has some agonist action at other 5-HT receptor types [59], while phenylbiguanide usually has a similar efficacy to 2-Me-5-HT but is inactive at some (e.g. guinea pig) 5-HT<sub>3</sub> receptors [60-61]. In contrast, *m*CPBG is 10-fold more potent than 5-HT, although it shows some partial agonist characteristics, in common with all the agonists described above [57]. These and other agonists are shown in Fig. (4), with EC<sub>50</sub> values shown in

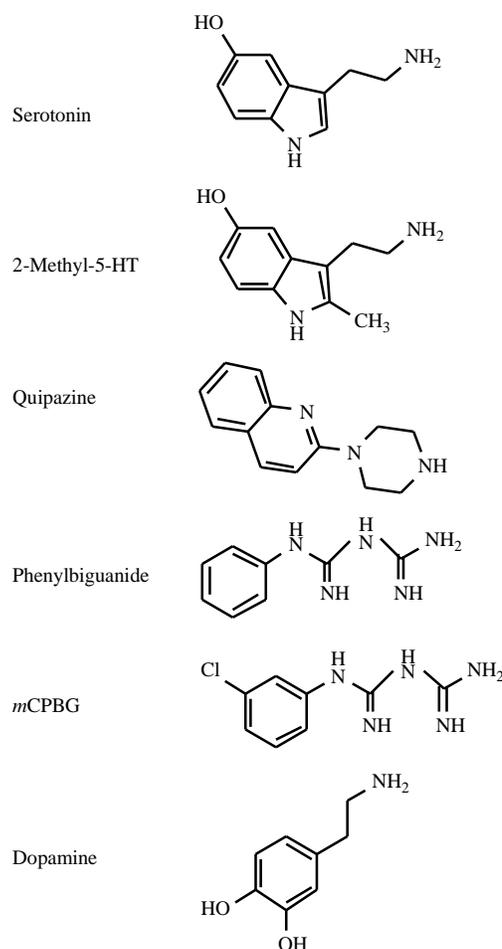


Fig. (4). Examples of 5-HT<sub>3</sub> receptor agonists.

Table 1. More recently developed agonists include arylbiguanides and arylguanides, with arylguanides having less lipid solubility than the corresponding arylbiguanides [58]. The introduction of a quinazoline ring and multiple chloro groups to the aromatic ring has been shown to increase the lipophilicity of both these compounds, demonstrating how

the construction of brain-penetrable analogs might be achieved in future [62]. *N*-methylquipazine (NMQ) has also been extensively manipulated by fusing aromatic or hetero-aromatic rings at various positions, which has resulted in a series of compounds with varied affinities [63-69]. The design of novel compounds has enabled the development of

**Table 1.  $K_i$  and  $EC_{50}$  Values for 5-HT<sub>3</sub> Receptor Agonists and Partial Agonists**

Agonist	$K_i$ or $EC_{50}$	Species	Reference
5-HT	1.8 $\mu M^y$	N1E-115	[146]
5-HT	1.56 $\mu M^y$	Mouse*	[39]
5-HT	123 nM	Human*	[137]
5-HT	219 nM	Rat tissue homogenate	[143]
2-Methyl-5-HT	11.0 $\mu M^y$	N1E-115	[142]
2-Methyl-5-HT	644 nM <sup>y</sup>	Human*	[2]
2-Methyl-5-HT	224 nM	Human*	[137]
2-Methyl-5-HT	562 nM	Rat tissue homogenate	[143]
Phenylbiguanide	1.8 $\mu M^y$	NG 108-15	[139]
Phenylbiguanide	10.1 $\mu M^y$	Human*	[2]
Phenylbiguanide	18 $\mu M^y$	Mouse <sup>§</sup>	[135]
Phenylbiguanide	2.4 $\mu M$	Human*	[137]
Phenylbiguanide	1.2 $\mu M$	NG 108-15	[58]
Phenylbiguanide	135 nM	Rat tissue homogenate	[143]
<i>m</i> CPBG	480 nM	Human*	[2]
<i>m</i> CPBG	400 nM <sup>y</sup>	Mouse <sup>§</sup>	[135]
<i>m</i> CPBG	4.77 nM	Rat tissue homogenate	[143]
<i>m</i> CPBG	19.5 nM	Human*	[137]
Quipazine	27 nM <sup>y</sup>	Human*	[2]
Dopamine	135 $\mu M^y$	Human*	[2]
<i>m</i> CPP	1.7 $\mu M^y$	Human*	[2]
Y-25130	36 nM <sup>y</sup>	Human*	[2]
2-chloro-phenylbiguanide	62 nM	NG 108-15	[58]
3-chloro-phenylbiguanide	17 nM	NG 108-15	[58]
4-chloro-phenylbiguanide	200 nM	NG 108-15	[58]
2-naphthylbiguanide	12 nM	NG 108-15	[58]
2-methoxy-5-chloro-phenylpiperazine	40 nM	NG 108-15	[58]
3, 4-dichlorophenylguanidine	3.1 nM	NG 108-15	[144]
4-biphenylguanidine	7 nM	NG 108-15	[144]
3,4,5-trichlorophenylbiguanide	0.7 nM	NG 108-15	[145]
3,4-dichlorophenylbiguanide	3.1 nM	NG 108-15	[145]

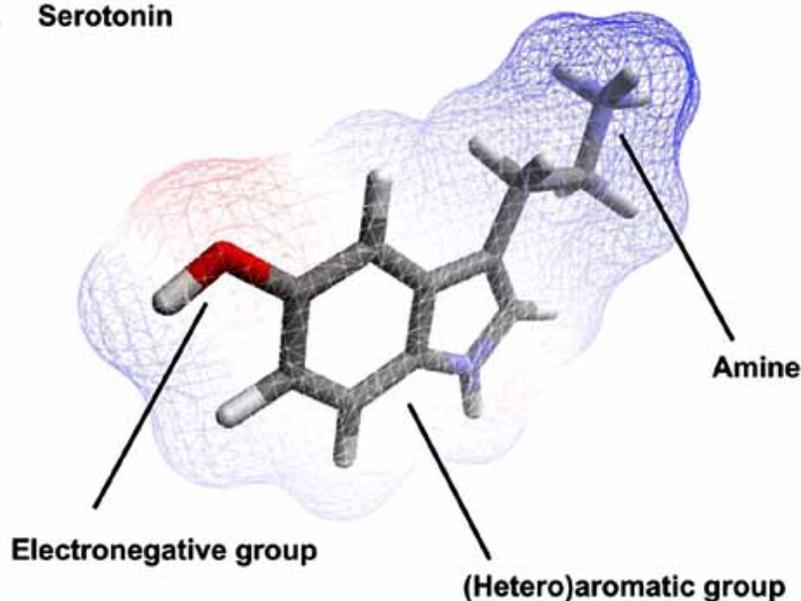
<sup>§</sup> Expressed in *Xenopus* oocytes; \* Expressed in HEK293 cells; \* Note that quipazine has been classified as both an agonist and non-selective antagonist; <sup>y</sup>  $EC_{50}$  values calculated using electrophysiological techniques.

pharmacophore models. One of the current pharmacophore models is shown in Fig. (5) and possesses a basic amine, an aromatic ring, a hydrophobic group and a hydrogen bond acceptor [66, 70, 71].

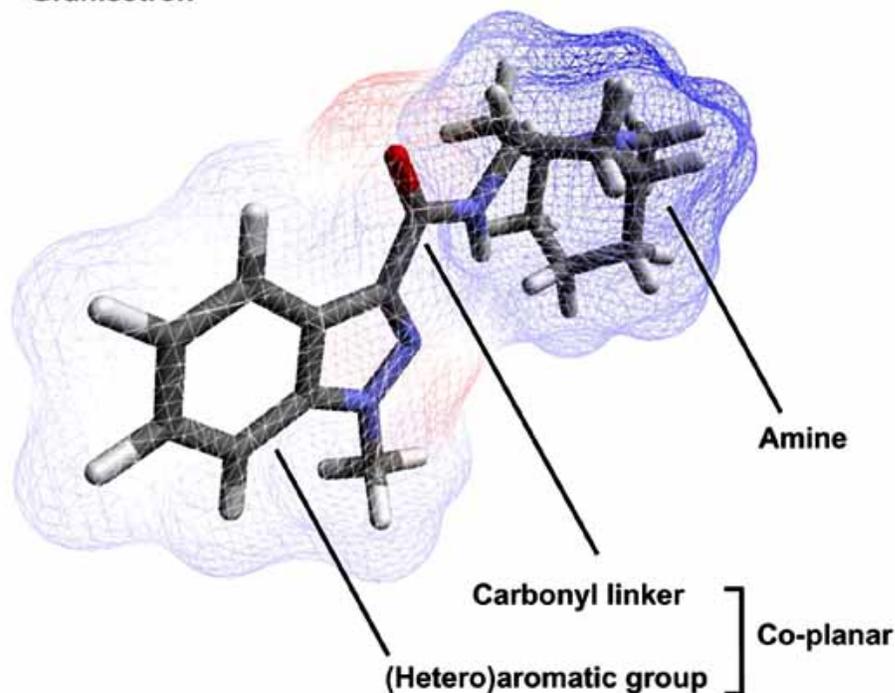
Studies of agonist binding to homology models of the 5-HT<sub>3</sub> receptor have identified amino acids that may be in-

involved in binding. Reeves *et al.* [41] found that 5-HT was orientated in the binding site with the charged primary amine located between the W183 and Y234 at the top of the binding site and the hetero-aromatic rings between W90 and F226 (Fig. (6)). This orientation is supported by mutagenesis data, and in particular by the evidence that W183 interacts with the primary amine via a cation- interaction [15, 46].

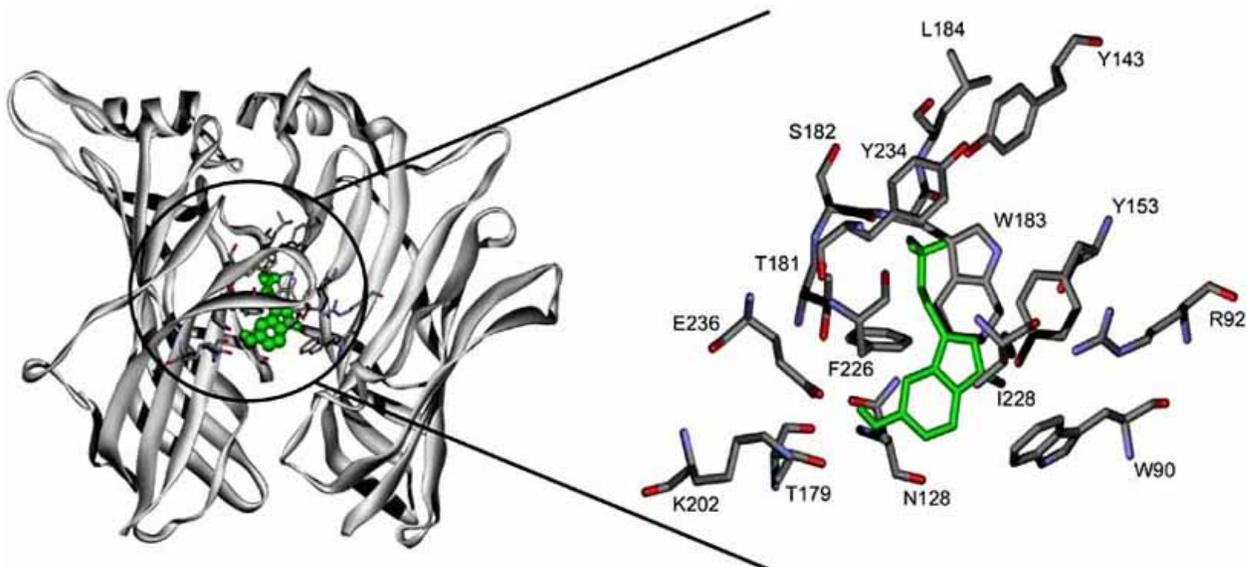
### 5A Serotonin



### 5B Granisetron



**Fig. (5).** 5-HT<sub>3</sub> receptor agonist and antagonist pharmacophores. 5-HT (Fig. 5A) and granisetron (Fig. 5B) are shown as examples of 5-HT<sub>3</sub> receptor agonists and antagonists. Electrostatic potential is displayed in wire-frame and shows negative potential in red and positive potential in blue. Attention has been drawn to the important features of each pharmacophore.

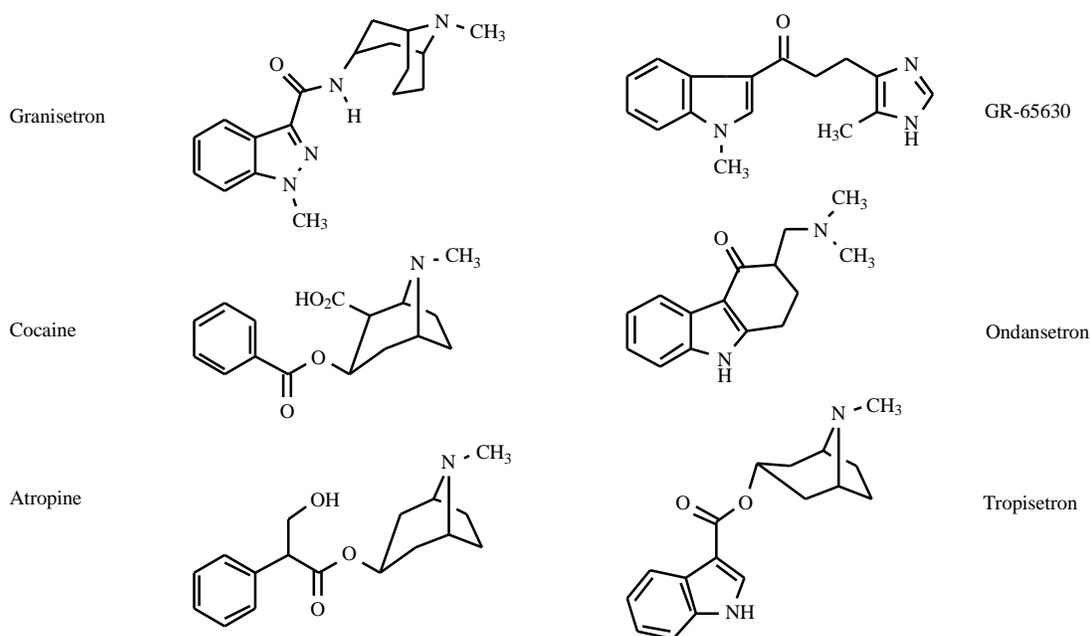


**Fig. (6).** 5-HT bound to the ligand-binding site of the 5-HT<sub>3</sub> receptor. Adapted from Reeves *et al.* 2003 (Model 4, [41]) where a more detailed description of the binding residues can be found.

### 5-HT<sub>3</sub> Receptor Antagonists

There are many highly selective and potent compounds that antagonise this receptor and a number of these are shown in Fig. (7). Early studies categorised the receptor using the non-selective compounds morphine and cocaine [72], but using 5-HT as the origin, bemesetron and tropisetron

were formulated. Further developments led to compounds that included ondansetron, granisetron and zacopride, which act at nanomolar concentrations, and there are now a wide range of similarly potent compounds (Tables 2 and 3). Biochemical comparisons and the physiological relevance of many of these compounds have been extensively investigated (e.g. [73, 74]).



**Fig. (7).** Examples of selective and non-selective 5-HT<sub>3</sub> receptor antagonists.

Table 2. K<sub>d</sub>, K<sub>i</sub> and IC<sub>50</sub> Values for 5-HT<sub>3</sub> Receptor Selective Antagonists

Selective Antagonist	K <sub>d</sub> , K <sub>i</sub> or IC <sub>50</sub>	Species	Reference
Tropisetron	11 nM <sup>‡</sup>	Human*	[2]
LY-278,584	5 nM <sup>‡</sup>	Human*	[2]
Y-25130	36 nM <sup>‡</sup>	Human*	[2]
Granisetron	230 pM <sup>‡</sup>	N1E-115	[136]
Granisetron	140 pM	Mouse <sup>§</sup>	[135]
Granisetron	1.44 nM	Human*	[137]
Granisetron	5.13 nM	Rat tissue homogenate	[143]
Tropisetron	46 pM <sup>‡</sup>	Rabbit nodose ganglion	[138]
Tropisetron	3.85 nM <sup>‡</sup>	NG 108-15	[139]
Tropisetron	4.9 nM	Rat tissue homogenate	[143]
Ondansetron	57 pM <sup>‡</sup>	Rabbit nodose ganglion	[138]
Ondansetron	440 pM <sup>‡</sup>	Mouse*	[140]
Ondansetron	7.4 nM <sup>‡</sup>	N1E-115	[141]
Ondansetron	0.25 nM <sup>‡</sup>	NCB-20	[6]
Ondansetron	4.9 nM	Human*	[137]
Ondansetron	46.8 nM	Rat tissue homogenate	[143]
Bemesetron	330 pM <sup>‡</sup>	Rabbit nodose ganglion	[138]
MDL-72222	3.5 nM <sup>‡</sup>	N1E-115	[142]
MDL-72222	16 nM <sup>‡</sup>	N1E-115	[141]
MDL-72222	30.2 nM	Rat tissue homogenate	[143]
BRL-46470	150 pM <sup>‡</sup>	Mouse <sup>§</sup>	[135]
BRL-46470	1.58 nM	Rat tissue homogenate	[143]
BRL-43694	230 pM <sup>‡</sup>	N1E-115	[141]
ICS-205-930	640 pM <sup>‡</sup>	N1E-115	[141]
Quipazine	1 nM <sup>‡</sup>	N1E-115	[141]
Quipazine	1.1 nM	Rat tissue homogenate	[143]
GR-65630	2.5 nM <sup>‡</sup>	N1E-115	[141]
SDZ 206-830	871 pM	Rat tissue homogenate	[143]
(S)-zacopride	955 pM	Rat tissue homogenate	[143]
(R)-zacopride	10.9 nM	Rat tissue homogenate	[143]
Alosetron	3.16 nM	Rat tissue homogenate	[143]
Renzapride	67.6 nM	Rat tissue homogenate	[143]
Clozapine	269 nM	Rat tissue homogenate	[143]
2-(4-methyl-1-piperazine)cyclohexa[c]quinoline	0.23 nM	Rat brain homogenate	[154]
Palonosetron	31.6 nM	Rat cerebral cortex, Rabbit ileal myenteric plexus, Guinea-pig ileal plexus	[152]
Dolasetron	20.03 nM	NG 108-15	[153]

<sup>§</sup> Expressed in *Xenopus* oocytes; \* Expressed in HEK293 cells; † Note that quipazine has been classified as both an agonist and non-selective antagonist; ‡ IC<sub>50</sub> values calculated using electrophysiological techniques.

**Table 3.  $K_i$  and  $IC_{50}$  Values for 5-HT<sub>3</sub> Receptor Non-Selective Antagonists**

Non-selective Antagonist	$K_i$ or $IC_{50}$	Species	Reference
Metaclopramide	12 nM <sup>‡</sup>	Rabbit nodose ganglion	[138]
Metaclopramide	50 nM <sup>‡</sup>	Mouse*	[140]
Metaclopramide	355 nM	Human*	[137]
Cocaine	83 nM <sup>‡</sup>	Rabbit nodose ganglion	[138]
Cocaine	3.4 $\mu$ M <sup>‡</sup>	Mouse <sup>§</sup>	[135]
Cocaine	2.45 nM	Rat tissue homogenate	[143]
(+)-Tubocurarine	160 nM	Rabbit nodose ganglion	[138]
(+)-Tubocurarine	1.3 nM <sup>‡</sup>	N1E-115	[146]
Atropine	2 $\mu$ M <sup>‡</sup>	N1E-115	[141]
Atropine	3.09 $\mu$ M	Rat tissue homogenate	[143]
QNB	7.6 $\mu$ M <sup>‡</sup>	N1E-115	[141]
Nicotine	53 $\mu$ M <sup>‡</sup>	N1E-115	[141]
-Bungarotoxin	>1 $\mu$ M <sup>‡</sup>	N1E-115	[141]
Methiothepin	>10 mM <sup>‡</sup>	N1E-115	[141]
Chlorpromazine	400 nM	N1E-115	[147]
Chlorpromazine	900 nM <sup>‡</sup>	N1E-115	[148]
Prochlorperazine	1.2 $\mu$ M	N1E-115	[147]
Perphenazine	1.5 $\mu$ M	N1E-115	[147]
Trifluoperazine	1.1 $\mu$ M	N1E-115	[147]
Fluphenazine	3.9 $\mu$ M	N1E-115	[147]
QX-222	29 $\mu$ M <sup>‡</sup>	N1E-115	[148]
Mepyramine	2.88 $\mu$ M	Rat tissue homogenate	[143]
Paroxetine	4.77 $\mu$ M	Rat tissue homogenate	[143]
Phentolamine	16.9 $\mu$ M	Rat tissue homogenate	[143]
Naloxone	195 $\mu$ M	Rat tissue homogenate	[143]
Propranolol	23.4 $\mu$ M	Rat tissue homogenate	[143]

<sup>§</sup> Expressed in *Xenopus* oocytes; \* Expressed in HEK293 cells; <sup>‡</sup>  $IC_{50}$  values calculated using electrophysiological techniques.

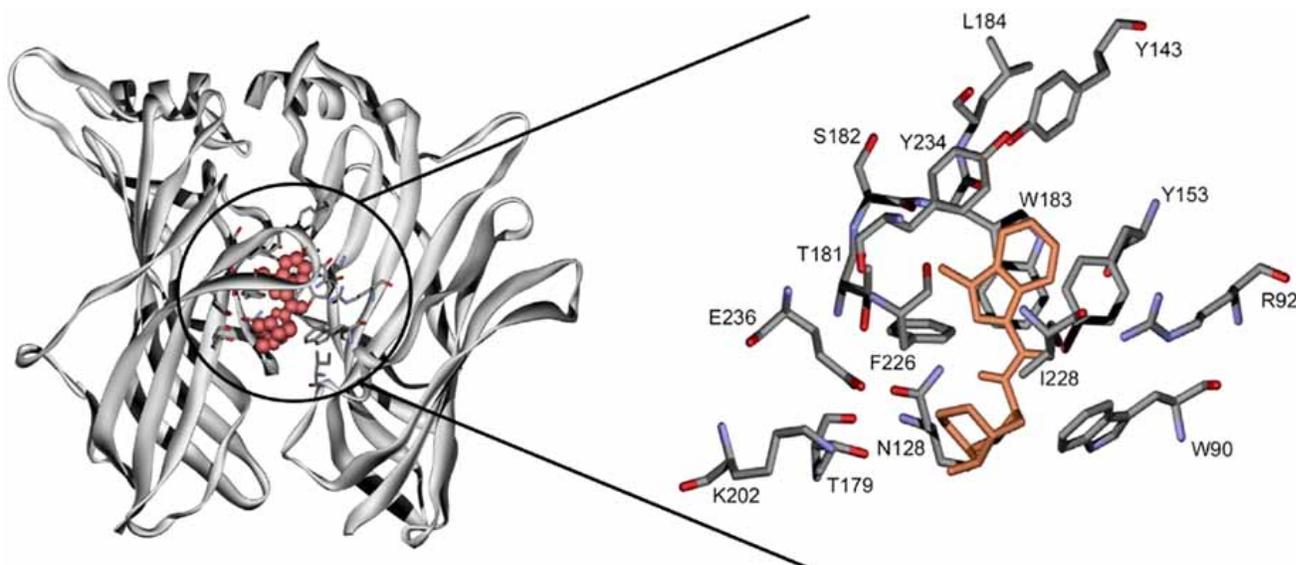
5-HT<sub>3</sub> receptor antagonists share a basic amine, a rigid aromatic or heteroaromatic ring system and a carbonyl group (or isosteric equivalent) that is coplanar to the aromatic system, and there are slightly longer distances between the aromatic and amine group when compared to the agonist pharmacophore (Fig. (5)). Further work has shown that the 5-HT<sub>3</sub> receptor can only accommodate small substituents on the charged amine, and a methyl group here appears to be optimal [75]. Most of the potent antagonists of 5-HT<sub>3</sub> receptors usually have 6, 5 heterocyclic rings and the most potent have an aromatic 6-membered ring.

Docking of a range of antagonists into a homology model of the 5-HT<sub>3</sub> receptor binding site shows a reasonably good agreement with the pharmacophore model and supports the observed differences between species [16, 42]. An example,

showing granisetron in the binding pocket, reveals the aromatic rings of granisetron lie between W183 and Y234 and the azabicyclic ring between W90 and F226 (Fig. (8)). Interestingly in this study another energetically favourable location of granisetron was identified, closer to the membrane, in a position that could be part of a binding/unbinding pathway for the ligand. A similarly located alternative binding site for granisetron has since been identified in another study of the 5-HT<sub>3</sub> receptor [76].

### 5-HT<sub>3</sub> Receptor Modulators

A wide range of substances, including divalent cations, alcohols, steroids and anaesthetics have been reported to modulate 5-HT<sub>3</sub> receptors, although their mechanisms of action are largely unknown. Modulation by divalent cations



**Fig. (8).** Granisetron bound to the ligand-binding site of the 5-HT<sub>3</sub> receptor. Adapted from Thompson *et al.*, (Model B, [16]) where a more detailed description of the binding residues can be found.

has been extensively investigated. For example, Ca<sup>2+</sup> acts as an inhibitor and probably acts both within the pore [77] and at the binding site [78], although its effects may be complicated by the finding that calcineurin potentiates receptor desensitisation [79, 80]. Mg<sup>2+</sup> and other divalent cations, including Cd<sup>2+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>, have also been shown to modulate 5-HT<sub>3</sub> receptor responses [8, 81]. Like Ca<sup>2+</sup>, these may act at multiple sites, although the effects of Zn<sup>2+</sup> appear more complex, with both inhibition and enhancement being reported [8, 82].

Alcohols also modulate 5-HT<sub>3</sub> receptors. Analyses of receptor kinetics show that they enhance the function of 5-HT<sub>3</sub> receptors by favouring the open state, although larger alcohols are inhibitory, with inhibition increasing with *n*-alkanol carbon chain length (see [83] for review).

Anaesthetics may manifest some of their effects through 5-HT<sub>3</sub> receptors. Halothane and isoflurane, for example, potentiate 5-HT<sub>3</sub> receptor-mediated responses, and there is also evidence of modulation by anaesthetics as diverse as etomidate, ketamine and methohexital (reviewed in [84]). Studies using the chimaeric 7-nACh-5-HT<sub>3</sub> receptor suggest that halothane and isoflurane, and therefore possibly other anaesthetics, act at the extracellular N-terminal domain [85]. Local anaesthetics may also act at 5-HT<sub>3</sub> receptors: procaine, tetracaine, bupivacaine, lidocaine, cocaine and QX222, have been reported to inhibit 5-HT<sub>3</sub> receptor function (see [86] for review).

There is also increasing evidence that steroids can affect the 5-HT<sub>3</sub> receptor. For example, the gonadal steroids 17 $\beta$ -estradiol and progesterone appear to act as non-competitive antagonists, with IC<sub>50</sub> values similar to those observed for their inhibitory action on glycine and nACh receptors [87]. The concentrations at which these compounds are effective exceed normal physiologically relevant levels but, as fluc-

tuation in gonadal steroids may underlie some behavioural disorders and nausea, for example during pregnancy, 5-HT<sub>3</sub> receptors have been proposed to provide a potential target for new drugs to treat these problems.

A range of other compounds also modulate 5-HT<sub>3</sub> receptor activity. The L-type Ca<sup>2+</sup> channel antagonists verapamil, diltiazem and nimodipine all inhibit 5-HT<sub>3</sub> receptor function [88], and a more detailed study on the action of diltiazem shows that it acts as a channel blocker [89]. Other compounds that inhibit 5-HT<sub>3</sub> receptors include tetraethylammonium,  $\alpha$ -conotoxin GVIIIA, phenothiazines, ifenprodil, bisindolylmaleimide and 5-hydroxyindole at high concentrations, although interestingly this latter compound reduces the rate of 5-HT<sub>3</sub> receptor desensitisation at low concentrations (see [86] for review). Overall there are many compounds that modulate 5-HT<sub>3</sub> receptors and some of these may have the potential to be useful as therapeutics.

## HETEROMERIC AND HOMOMERIC RECEPTORS

The presence or absence of the 5-HT<sub>3B</sub> subunit explains why reports of 5-HT<sub>3</sub> receptors electrical properties differ. For example, the current-voltage relationship has been described as both linear and inwardly rectifying and there also have been controversies about the single channel conductance and Ca<sup>2+</sup> permeability of native receptors [1, 9]. In heterologously expressed receptors, these properties are significantly affected by the presence of 5-HT<sub>3B</sub> receptor subunits providing convincing evidence that at least some native 5-HT<sub>3</sub> receptors possess such subunits. Given there are significant differences in the amino acids that constitute that binding pocket in homomeric 5-HT<sub>3A</sub> versus heteromeric 5-HT<sub>3A/B</sub> receptors, it is surprising that the pharmacological profile of heteromeric and homomeric receptors is broadly similar [2, 90]. It may be that agonists and antago-

nists can only bind when two A subunits are adjacent, although evidence suggests that the stoichiometry in heteromeric receptors is BBABA [91]. Nevertheless, some pharmacological differences have been reported. 5-HT<sub>3A/B</sub> heteromers have reduced sensitivity to picrotoxin and their enhancement of channel gating by some anaesthetics (especially halothane and chloroform) is markedly attenuated [92, 93]. These differences could have important implications for drug therapy.

## THERAPEUTIC POTENTIAL

Studies have suggested many diverse potential disease targets that might be amenable to alleviation by 5-HT<sub>3</sub> receptor selective compounds, including addiction, pruritus and neurological phenomena such as anxiety, psychosis, nociception and cognitive function [94-99]. However, the main therapeutic targets are currently irritable bowel syndrome (IBS) and emesis resulting from cancer chemotherapy [100, 101]. Each potential therapeutic application is discussed in more detail below.

The theory of 5-HT involvement in schizophrenia and bipolar disorder was first presented in 1954 and it proposed that there was a serotonergic deficiency in schizophrenic individuals [102, 103]. 5-HT<sub>3</sub> receptors are prime candidates due to their functional diversity and their ability to modulate the release of other neurotransmitters such as dopamine. To date the focus of 5-HT and its impact on schizophrenia has been on the G-protein coupled 5-HT<sub>2</sub> receptors, but drugs such as ondansetron and clozapine, which have positive effects on schizophrenic symptoms, are both 5-HT<sub>3</sub> receptor antagonists [104, 105]. There are also reports of mutations in 5-HT<sub>3</sub> receptor sequences in patients with bipolar disorder and schizophrenia, although there is currently no evidence these play a role in the pathology of the disease [106, 107].

There is some experimental evidence to suggest that the 5-HT<sub>3</sub> receptor plays a role in a variety of psychological disorders, although the use of 5-HT<sub>3</sub> receptor antagonists has met with little success. The deletion of the 5-HT<sub>3</sub> receptor gene creates knockout mice that exhibit anxiolytic behaviour [108, 109] and the use of 5-HT<sub>3</sub> receptor antagonists has shown a range of anxiolytic effects [110]. Interestingly, a variety of antipsychotic drugs are non-competitive 5-HT<sub>3</sub> receptor antagonists and this functional antagonism may contribute to their antipsychotic efficacy [111].

The use of 5-HT<sub>3</sub> receptor antagonists has also been employed for the alleviation of substance abuse. Antagonists are particularly effective at reducing ethanol and morphine self-administration, but are less effective at reducing the self-administration of psychostimulants such as cocaine [112-115]. Interestingly, it has been shown that with the administration of ondansetron, alcohol craving is significantly reduced in early onset alcoholics, but increased in late onset alcoholics [96]. Substance abuse is particularly high among patients suffering from schizophrenia, suggesting a possible link between the systems that modulate these responses [116, 117].

It has been suggested that 5-HT<sub>3</sub> antagonists may have an antipruritic effect [118, 119]. However, current research reveals mixed reports in this area, and the effectiveness of

treatment may vary according to the type of pruritus studied. For example, in patients with cholestatic itch, either some or no benefit has been reported [120, 121], whilst only marginal or no relief has been reported for hemodialysis-related pruritus [122, 123]. However, the underlying mechanisms of this disorder are still poorly understood and will need further work if a therapeutic potential is to be realised [124].

The best established clinical use of 5-HT<sub>3</sub> receptor antagonists is for chemotherapy induced emesis, and they can be particularly effective when used in combination with other therapies [125, 126]. There is some evidence to suggest that the 5-HT<sub>3B</sub> receptor subunit plays an important contribution to the effectiveness of these compounds, and, given its location, this is a reasonable hypothesis. A study of polymorphisms showed a positive link between a mutation in the promoter region of the 5-HT<sub>3B</sub> gene and the frequency of vomiting [127], but in other studies no link between polymorphisms and pharmacological responses has been made [128, 129].

The second major clinical application of 5-HT<sub>3</sub> receptor antagonists is in the treatment of irritable bowel syndrome. For example, the 5-HT<sub>3</sub> antagonist alosetron, has been particularly widely used and has been found to decrease gut transit [96], increase fluid absorption [130] and reduce pain [131-133]. The role of 5-HT<sub>3</sub> receptors in this syndrome has been reviewed by Gershon [134].

## SUMMARY

5-HT<sub>3</sub> receptors are well understood in terms of their distribution, structure, function and pharmacology. However, there is still some way to go in order to understand their roles in both the CNS and the PNS and a better knowledge of this might lead to more areas for therapeutic intervention by 5-HT<sub>3</sub> receptor agonists, antagonists and modulators. Currently 5-HT<sub>3</sub> receptor antagonists are proving to be useful agents for controlling chemotherapy induced emesis and in irritable bowel syndrome, but as studies suggest there is considerable potential for therapeutic intervention in other areas, we anticipate that there will be further developments in their clinical use.

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## ABBREVIATIONS

AChBP	=	Acetylcholine binding protein
nACh receptor	=	Nicotinic acetylcholine receptor
LGIC	=	Ligand gated ion channel

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