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Nucleotide Contribution to the Functioning of SERT, Na^+/K^+ ATPase and GPCR Proteins

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Abstract

Purine nucleotides are crucial for the effective operation of cell membrane proteins maintaining the neurotransmitter responses of 5-HT. Major protein targets in the treatment of depression include SERT, N/K ATPase and GPCR. Each protein target is responsive to a specific complement of drugs: antidepressants (SERT), lithium and cardiogenic steroids (N/K ATPase), 5-HT receptor ligands (GPCR). Computational software is useful for comparing molecular similarity within ligand-ligand and ligand-nucleotide structures. Previous studies demonstrate that GPCR ligands of different pharmacologic classes display relative molecular similarity to nucleotide structures. The current study applies this methodology to compound structures modulating SERT and N/K ATPase receptors. Minimum energy conformers of SERT antagonists demonstrate relative molecular similarity to the structural template of GTP nucleotide. GTP template fits of 5-HT and psilocin are similar, whereas a SERT-like fit is one of several for the ketamine structure. Endogenous and pharmaceutical modulators of Na/K ATPase relate to adenine nucleotide. The fits of cardiogenic steroids to a cGMP template demonstrate similarities and differences between compounds. Relative molecular similarity within the structures of hormones, drugs and nucleotides has implications for neurotransmitter transport and cell signal transduction processes.

Keywords

SERT, SSRI, GPCR, Sodium/Potassium ATPase, Nucleotides, Depression

1. Introduction

Therapeutic advances for depressive disorders have permitted a shift in focus from the use of tricyclic antidepressant (TCA) to SSRI (selective serotonin reuptake inhibitor) medication. Lithium remains a long-standing treatment for pa-

tients with bipolar disorder (BPD) or those requiring augmentation of medication [1]. Patient management problems, associated with therapeutic response times and refractory depression, have more recently stimulated interest in the use of ketamine and natural products based on psilocybin; both increase neurotransmitter levels in rat frontal cortex [2]. Progress in understanding the complex role of serotonin (5-HT) in maintaining mental health lags behind demonstration of the transmitter's properties in the laboratory setting [3]. In reviewing the clinical effects of 5-HT, Carhart-Harris & Nutt [4] consider two major serotonin brain transmission pathways based on 5-HT_{1A} and 5-HT_{2A} receptor subtypes; the former enhanced by SSRIs and the latter by psychodelic agonists. Regulation of the SERT (pre-synaptic plasma membrane serotonin transporter associated with Na^+ and Cl^-) 5-HT transporter, however, involves the 5-HT_{2B} receptor [5].

SERT facilitates sodium- and chloride-dependent reuptake of neuron released 5-HT. SSRIs maintain tonic synapse concentrations of 5-HT by reducing removal via the protein transporter [5]. Central and vestibular binding sites on SERT are named respectively S1 and S2, with the latter designated as an allosteric site [6]. During the transport process, conformational changes in SERT alternately expose the central binding-site to extracellular or cytoplasmic media for substrate-binding or release [6]. Substrate and antagonists both target the primary binding-site of SERT. SERT proteins partition within plasma membrane lipid rafts; conformation and function are influenced by cholesterol [5]. Mutations in SERT are associated with psychiatric disorders and autism. Some mutations impacting on SERT activity influence the cGMP dependent phosphorylation of specific amino acid residues, a process initiated by conformational changes within the protein [7].

Sodium ions initiate the cycle of conformational change within the SERT protein, which is an energy requiring process driven by the plasma membrane sodium/potassium ATPase (N/K ATPase) [8]. Although there is evidence of sodium channel inhibition by SSRIs [9], lithium is the antidepressant most strongly associated with N/K ATPase modulation. Lithium normalises N/K ATPase activity and lipid peroxidation in blood samples from patients with BPD, and brain tissues of stressed rats [10] [11]. Lithium also augments 5-HT neurotransmission and SSRI treatment in animal and clinical settings [12]. Several endogenous compounds modulate 5-HT release from neurons. Histamine action (pre- and post-synaptically) inhibits 5-HT release; H_3 receptor antagonists are anxiolytic and antidepressive [13]. Thyronines show distinctive distribution patterns within the brain and allosteric effects on neurotransmitter receptors [14]. The N/K ATPase of the renal proximal tubule has an extracellular binding domain for cGMP, which inhibits Na^+ transport in a manner similar to the effects of ouabain [15]. Kidney medulla ATPase activity is modulated by fatty acid and acylglycerol compounds [16].

In common with other neurotransmitters, 5-HT signaling is regulated intra-

cellularly by G-proteins [17]. GTP nucleotide has a central role in the GPCR (G-protein coupled receptor) signaling process, which requires the cyclical regeneration of GTP-bound α proteins (Gα proteins). The Gα_s protein induces cAMP generation, a nucleotide in brain tissue characterised by low levels in depressed patients and increased levels following antidepressant treatment [18] [19]. There has been increasing interest in the dimerisation of GPCR receptors in past decades, to the extent that this state may be considered as the norm for the expression and function of GPCRs [17]. Purine nucleotides therefore participate in regulating the responses of SSRI, SERT and N/K ATPase proteins and 5-HT signaling. Previous studies demonstrate molecular similarity within the structures of agonists and antagonists of different receptor classes, relative to the nucleotides of guanosine and adenosine. The present work seeks to extend this observation to the above modulators of depression with the aim of consolidating knowledge in regard to their mechanism of action.

2. Material and Methods

2.1. Compound Structures

The compounds under investigation are SSRIs and inhibitors of SERT, as listed by the IUPHAR/BPS 2023. Guide to Pharmacology (https://www.guidetopharmacology.org). Additional compounds include the muco-active compound ambroxol, identified as an inhibitor of SERT in guinea pig colon [20]; ibogaine is described as an active site-binding non-competitive inhibitor of SERT [21]; MK-7145 is marketed by MedChemExpress (MCE; NJ 08852, USA) as a human SERT inhibitor in transinfected HEK293 cells. The chemicals for labelling SERT include ADAM ((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine) a high affinity SPECT (single photon emission computed tomography) tracer [22] and ASP (dimethylamino)styryl]-n-methylpyridinium) a fluorescent transporter substrate [23]. Arachidonic acid, monolaurin and dioctanoylglycerol are modulators of Na/K ATPase activity [16]. Compounds structures are taken from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/).

2.2. Molecular Modeling

Compound structures are built from contents of the Nemesis software program fragment file (Oxford Molecular version 2.1) and minimised by conformational analysis. The molecular structures used for fitting are minimum energy conformers in an uncharged form. The conformation of the cGMP structure is described by the torsion angle (bond angle described by 4 adjacent atoms) C8N9C1'O9 −33˚ (**Figure 1**). The same torsion angle in the GTP and ATP structures are respectively −47˚ and −38˚. The Nemesis program fits paired molecular structures on a three-point basis. Fitting-points, comprised of atoms of similar type and partial charge within compound and nucleotide structures, are identified in the text and table with respect to the nucleotide labels. Colour-coded atoms in the

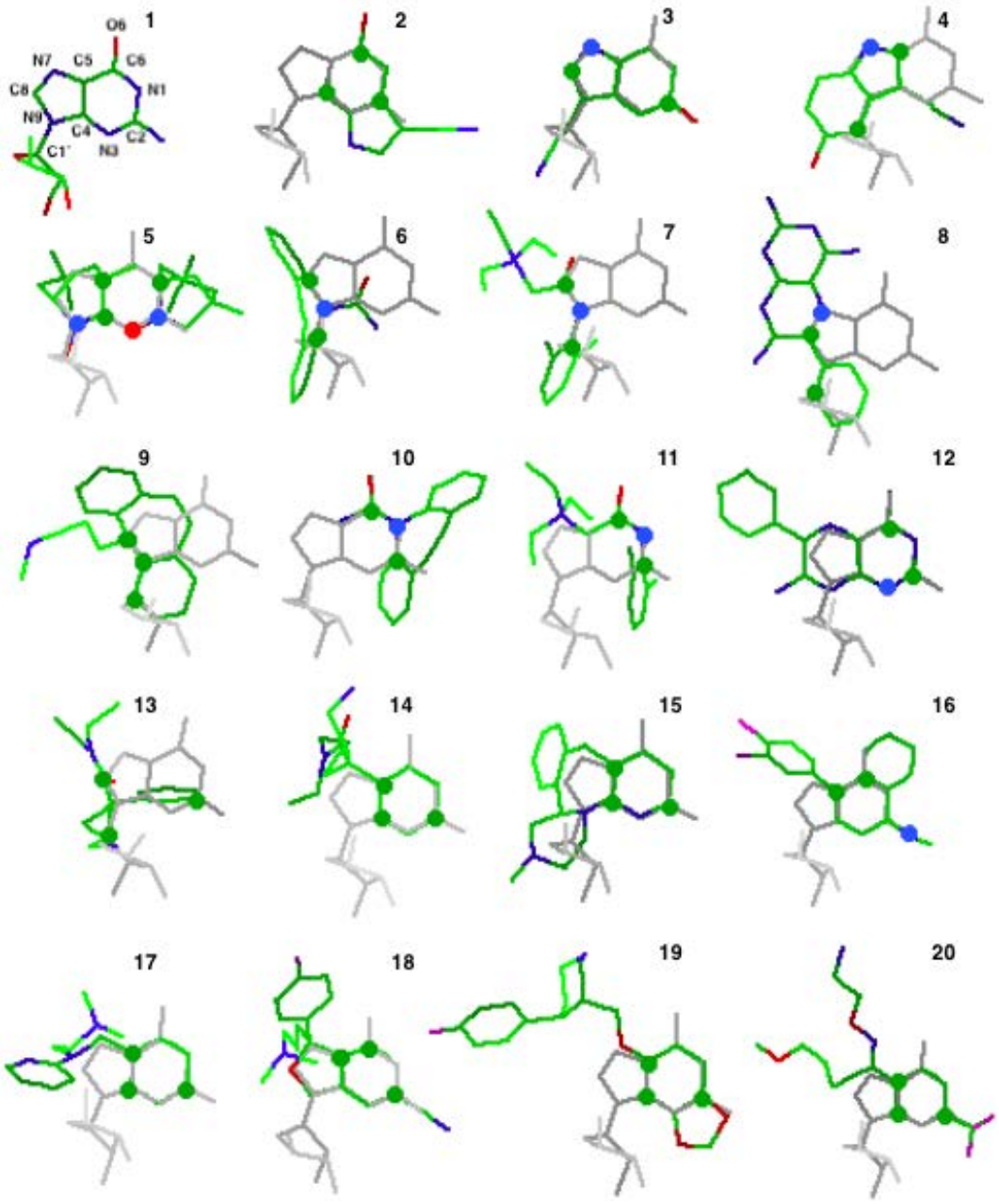

Figure 1. Fits of serotonin and SSRI structures to GTP template (grey). 1: GTP, 2 - 4: serotonin, 5: trazon, 6: carbamazepine, 7: QX314, 8: triamterene, 9: protriptyline, 10: carbamazepine, 11: QX314, 12: triamterene, 13: levomilnaipran, 14: levomilnaipran, 15: mirtazapine, 16: sealdin, 17: tripelennamine, 18: escitalopram, 19: paroxetine, 20: fluvoxamine.

figures identify ligand fitting-points: carbon-green, nitrogen-blue, oxygen-red, sulphur-yellow. To improve on presentation of the fitted compounds, bond order within molecular structures is not shown and the triphosphate chain of GTP is cropped. The Nemesis program computes goodness-of-fit values, in respect of inter-atomic distance at each fitting-point and root mean square (RMS) value. The sequence of fitting points for each structure (given in Table 1, left to right) provides the fit with the lowest RMS value.

Table 1. Values for fitting compound structures to nucleotide templates.

Compound	Nucleotide	Fitting points	Interatomic distance (Å)	RMS (Å)
5-HT	GTP	C2C4C6	0.06, 0.07, 0.01	0.0049
5-HT	GTP	C8N7C2	0.05, 0.02, 0.05	0.0022
5-HT	GTP	N7C5C1’	0.01, 0.00, 0.01	0.0002
ADAM	GTP	C2C4C5	0.05, 0.06, 0.02	0.0068
ambroxol	GTP	C2C4C5	0.06, 0.04, 0.03	0.0010
arachadonic acid	ATP	C5C1C2’	0.11, 0.12, 0.05	0.0033
bufalin	cGMP	C4C2’O9	0.04, 0.03, 0.07	0.0066
carbamazepine	GTP	C8N9C1	0.04, 0.05, 0.06	0.0112
carbamazepine	GTP	C6N1C2	0.01, 0.01, 0.02	0.0021
cocaine	GTP	C2C4C5	0.06, 0.04, 0.03	0.0010
cocaine	cGMP	C6N1C1’	0.06. 0.07, 0.06	0.0001
dapoxetine	GTP	C2C4C5	0.07, 0.06, 0.03	0.0056
digitoxigenin	cGMP	O9C1’C4	0.04, 0.09, 0.08	0.0159
1,2-dioctanoylglycerol	ATP	C1’N9C5	0.02, 0.09, 0.10	0.0023
escitalopram	GTP	C5C6C2	0.02, 0.03, 0.04	0.0003
fluvoaxamine	GTP	C2C4C5	0.07, 0.04, 0.04	0.0017
ibogaine	cGMP	C4’C3’N1	0.06, 0.05, 0.02	0.0006
ibogaine	GTP	C2C4C5	0.04, 0.04, 0.01	0.0013
immepip	GTP	C8N1C2	0.02, 0.04, 0.06	0.0042
(S)-ketamine	GTP	N9C1’C2’	0.05, 0.08, 0.04	0.0167
(S)-ketamine	GTP	C4N9C2’	0.10, 0.07, 0.03	0.0046
(S)-ketamine	GTP	O6C6C5	0.01, 0.05, 0.06	0.0052
(S)-ketamine	GTP	N1C2N3	0.07, 0.09, 0.03	0.0086
(S)-ketamine	GTP	C1’N9C8	0.05, 0.06, 0.03	0.0109
(S)-ketamine	GTP	C5C4C2	0.04, 0.03, 0.06	0.0004
(S)-ketamine	GTP	C2’C1’N9	0.05, 0.08, 0.05	0.0018

Continued

levomilnacipran	GTP	C2'C1'C8	0.03, 0.05, 0.07	0.0136
levomilnaipran	GTP	C2C4C5	0.06, 0.04, 0.02	0.0000
methylene blue	GTP	C2C4C5	0.07, 0.05, 0.04	0.0035
mirtazapine	GTP	C5C4C2	0.02, 0.01, 0.02	0.0007
MK-7145	GTP	C2C4C5	0.03, 0.03, 0.02	0.0037
1-monolauroylglycerol	ATP	C1'N9C5	0.02, 0.09, 0.09	0.0009
ouabain	cGMP	O9C2'C4	0.06, 0.05, 0.03	0.0016
ouabain	cGMP	N9C1'O8	0.11, 0.02, 0.10	0.0052
ouabain	cGMP	C2'C1'N9	0.08, 0.04, 0.10	0.0143
ouabain	GTP	N2C6C4	0.06, 0.09, 0.13	0.0071
paroxetine	GTP	C2C4C5	0.04, 0.05, 0.02	0.0072
propafenone	ATP	O9C1'C8	0.05, 0.01, 0.04	0.0109
protriptyline	GTP	C8N9C1'	0.08, 0.08, 0.01	0.0019
psilocin (13)	GTP	C5C4C2	0.02, 0.03, 0.04	0.0019
psilocin (14)	GTP	C5C4C2	0.05, 0.03, 0.08	0.0016
psilocybin	GTP	C5C4C2	0.02, 0.04, 0.03	0.0048
QX314	GTP	C8N9C1'	0.05, 0.02, 0.06	0.0077
QX314	GTP	C6N1C2	0.02, 0.01, 0.01	0.0017
rostafuroxin	cGMP	C4C2'O9	0.02, 0.05, 0.06	0.0104
sealdin	GTP	C5C6N2	0.10, 0.05, 0.09	0.0029
triiodothyronine (T3)	ATP	C5C6C3'	0.05, 0.07, 0.05	0.0066
trazon	GTP	N3C2N1	0.07, 0.01, 0.08	0.0058
trazon	GTP	N9C4C5	0.02, 0.07, 0.07	0.0075
thioperamide	GTP	C2N3N9	0.03, 0.07, 0.04	0.0120
triamterene	GTP	N7C8C1'	0.03, 0.03, 0.02	0.0072
triamterene	GTP	C6C2N3	0.03, 0.03, 0.04	0.0039
tripelennamine	GTP	C5C4C2	0.02, 0.04, 0.05	0.0040
vortioxetine	GTP	C2C4C5	0.06, 0.05, 0.02	0.0042

3. Results

Three fits of the minimum energy 5-HT conformer to the GTP structure are given in Figure 1 (2 - 4), followed by those of compounds regarded as sodium channel blockers (6 - 12). Protriptyline and carbamazepine are tricyclic compounds with antidepressant and anticonvulsant uses, respectively. QX314 has local anaesthetic properties, whereas triamterene is a diuretic. These drug structures have fitting-points on the imidazole and ribose rings of the GTP template (6, 7, 8) and a second fit on the oxopyrimidine moiety involving C6, N1, C2/N3 atoms (10, 11, 12). Trazon (5), a SSRI and antidepressant, has a structure small enough to provide two non-overlapping fits on the guanine ring. One feature common to the remaining SSRI structures (13 - 20) and the previous antagonist structures in this figure, is the presence of substantial substituent groups of cyclic rings or alkyl-amino sidechains, in the 9 - 11 o'clock position with respect to imidazole atom N9. Distances between fitting-point C2 and the distal substituent atom range from 6.9Å (mirtazapine) to 11.8Å (paroxetine), with ADAM, sealdin, dapoxetine, ambroxol and fluvoxamine of the fitted structures providing values in-between, in ascending order. In contrast, the fitted SERT substrate (5-HT) has no bulky structure to extend into the space occupied by the SSRI inhibitors. The fit of one SSRI/SERT inhibitor thus covers approximately the same area as the double fit of a sodium channel blocker structure.

Figure 2 extends the number of SSRI/SERT structures fitted on the GTP template (1 - 7). As in the previous figure, the structures feature a bulky substituent group in the 9 - 11 o'clock position. ADAM is a tracer compound with high affinity for SERT, whereas vortioxetine is in use as an antidepressant. A SSRI/SERT-like fit is also given for ouabain (8). Immepip (9) and thioperamide (10), respectively H_3/H_4 receptor agonist and antagonist compounds, provide fits to the GTP template that differ from the SSRI/SERT compounds in that their imidazole rings provide only 2 fitting points on the nucleotide oxopyrimidine ring. The fits of methylene blue (11) (an antidepressant and guanylate cyclase inhibitor) and the fluorescent substrate ASP are similar to those of the SSRI/SERT inhibitors. Template 12 gives the superimposition of ASP on the methylene blue structure (grey), demonstrating their similarity. Fitting-point values of SSRI/SERT inhibitor structures on GTP are mostly less than 0.1Å, (Table 1) and RMS values are <0.02Å. The nucleotide template fits of psilocin and psilocybin structures (13 - 15) are more similar to those of 5-HT. In keeping with the disparate properties of ketamine, the minimum energy conformer demonstrates several fits to the GTP template (16 - 22), including one (21) comparable to those of SSRI/SERT inhibitors.

The SERT transporter operates using energy produced by Na/K ATPase activity. Compound structures in Figure 3 (1 - 5) fit to a low energy conformer of ATP that illustrates the potential for interaction with the the nucleotide triphosphate chain, substrate of the ATPase. Arachidonic acid (1), propafenone (2) and triiodothyronine (T3) (3) are activators of Na/K ATPase. Monolauroylgly-

cerol (4) is also an activator, whereas dioctanoylglycerol (5) is an inhibitor of the ATPase. cGMP, a nucleotide regulator of Na/K ATPase, provides the fitting template for the structures of ibogaine (6), cocaine (7) and cardiac steroids (8 - 12). Fits of the inhibitor ouabain (8, 9), based on the steroid nucleus, are replicated by bufalin (11), rostafuroxin (12) and digitoxigenin (not shown). These steroid structures do not replicate the ouabain fit of template 10 because they lack a sugar moiety. Template 13 demonstrates relative molecular similarity in a composite fit (based on template 8) of the 4 steroid structures without the nucleotide. The fitting data of compound structures in Figure 3 are comparable to those of the SSRI/SERT inhibitors (Table 1).

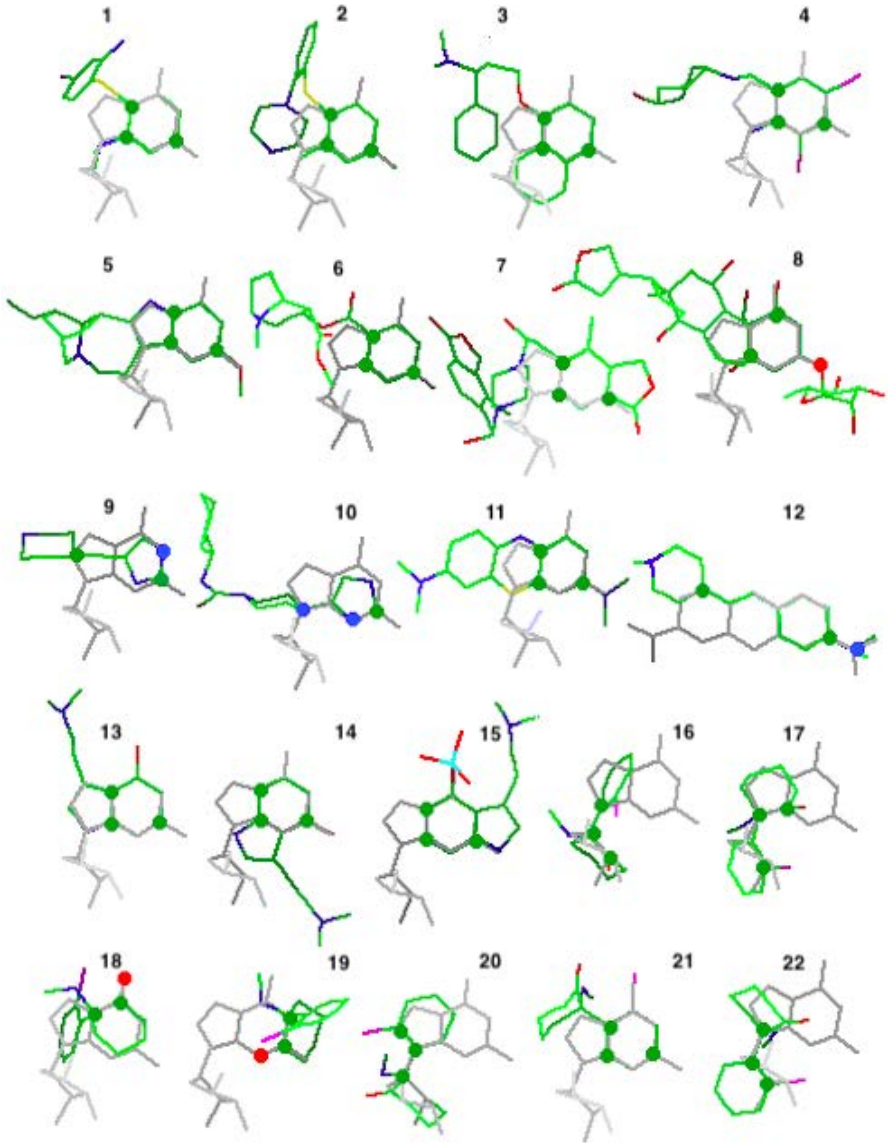

Figure 2. Fits of SSRI and SERT inhibitor structures to GTP template (grey). 1: ADAM, 2: vortioxetine, 3: dapoxetine, 4: ambroxol, 5: ibogaine, 6: cocaine, 7: MK-7145, 8: ouabain, 9: immepip, 10: thioperamide, 11: methylene blue, 12: fit of ASP to methylene blue (grey), 13: psilocin, 14: psilocin, 15: psilocybin, 17 - 22: (S)-ketamine.

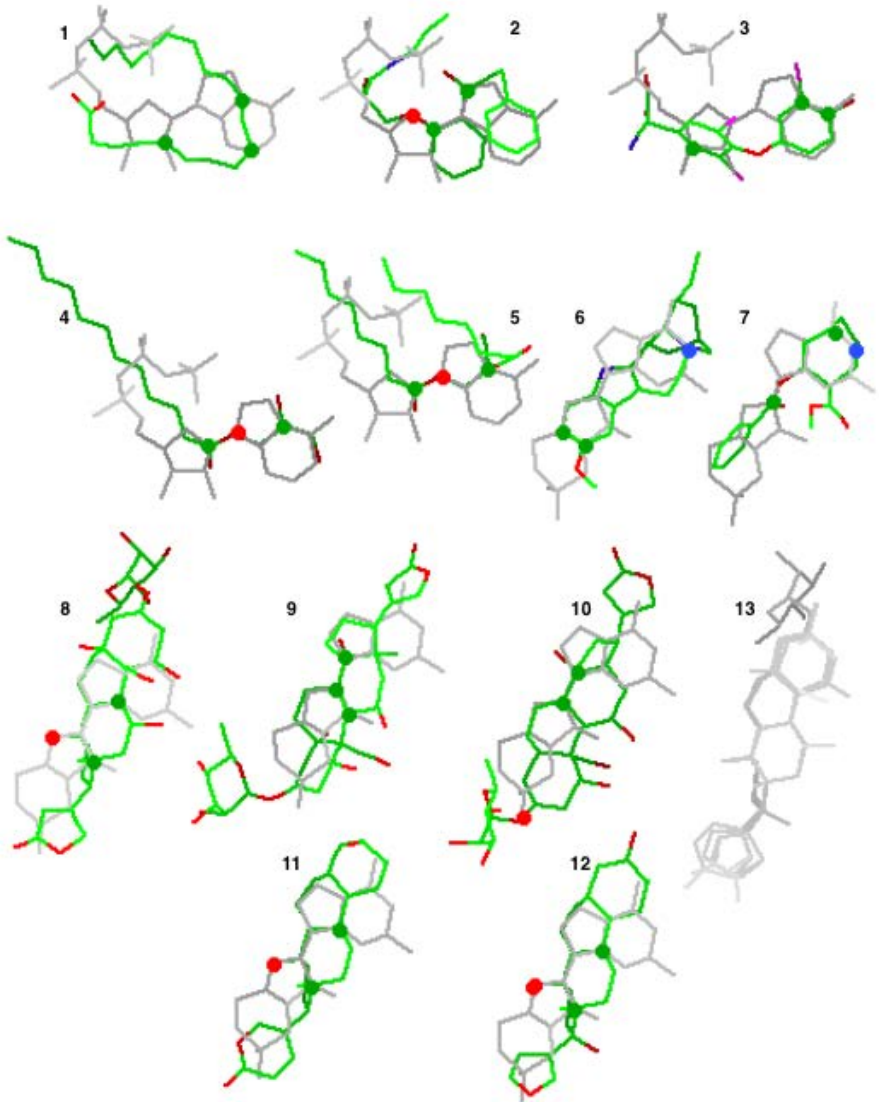

Figure 3. Fits of ATPase modulator structures to ATP template (grey) 1 - 5, and cGMP template (grey) 6 - 12. 1: arachidonic acid, 2: propafenone, 3: triiodothyronine, 4: monolauroylglycerol, 5: dioctanoylglycerol, 6: ibogaine, 7: cocaine, 8 - 10: ouabain, 11: bufalin, 12: rostafuroxin, 13: composite fit of ouabain, bufalin, rostafuroxin and digitoxigenin.

4. Discussion

SERT inhibitor structures demonstrate molecular similarity to GTP nucleotide, in respect of the fit of an aromatic or cyclic ring and the distant positioning of bulky substituent groups that do not contain the template fitting-points. The structures of tricyclic sodium channel blockers relate to the GTP template but with a different fitting pattern. Baudry and co-authors [5] describe the concentration dependent uptake of 5-HT by SERT via the 5-HT_{2B} receptor, regulated by NOS/PKG (low 5-HT) or PKC (high 5-HT) signalling processes and phosphorylation. Both SERT and N/K ATPase proteins are subject to a phosphorylation process, which is reproduced *in vitro* by the relevant second messengers [24]. Hyperphosphorylation of these proteins reduces 5-HT uptake and antidepres-

sant binding to SERT. SERT phosphorylation and transport activity is rapidly regulated *in vitro* by agonists and antagonists of protein kinases, notably PKC, PKG, and p38MAPK. The 5-HT_{2B} receptor controls the phosphorylation sites of SERT and N/K ATPase and the change in default coupling from PKG to PKC, resulting in hyperphosphorylation, provides an important intervention target for correction [5]. On the outward-open conformation of SERT an assembly complex of substrate and bound Na^+ initiates uptake of 5-HT; transport is not sustained by other monovalent ions [8]. The molecular structures of antidepressants wedge between the membrane-anchored scaffold domain and the mobile domain of outward-facing SERT [25]. Cocaine and Na^+ stabilise the outward-open conformation of SERT and decrease phosphorylation, whereas compounds that stabilise inward-open conformations (5-HT and ibogaine) increase phosphorylation. The differences between the template-fitted substrate and inhibitor structures suggest that the bulky substituent groups of the latter are responsible for preventing the occlusion of SERT, which is a necessary first step in the transporter process. The IUPHAR/BPS database lists protriptyline as an inhibitor of SERT whereas tripelennamine and mirtazapine are listed as SSRI drugs. Escitalopram, paroxetine and fluvoxamine are listed as both SERT and SSRI drugs. This class distinction between SERT inhibitors and SSRI is of unlikely significance.

Histamine H_3/H_4 receptor agonists reduce SERT phosphorylation and activity in rat hippocampal synaptosomes [13]. Antagonism by thioperamide and the failure to repeat these observations in cells devoid of histamine receptors indicates that histamine modulates 5-HT function on 5-HT neurons. In regard to the nucleotide template fits of these compounds, the imidazole ring of histamine derivatives appears to be a sufficient replacement for a six-membered carbon ring, evident in the psilocin and psilocybin template fits to 5-HT. Psilocin is a SERT inhibitor in rat brain synaptosomes but has greater affinity as an agonist of 5-HT_{2A} receptors [26]. A recent systematic review of psilocybin treatment of depression reports a lack of consensus between studies [27]. Ketamine inhibition of 5-HT transport is lost in a mouse strain lacking the transporter protein [28]. Positron emission tomography measurements in monkeys following sub-lethal doses of ketamine identify transient inhibition of SERT activity [29]. Ketamine promotes plasticity in the hippocampus, via a negative synaptic feedback response expressed by a subtype of AMPA receptor [30]. Inhibition of NMDA receptors on GABAergic interneurons leads to disinhibition and activation of AMPA receptor pathways [31]. The pleiotropic nature of ketamine [32] is evident in this study by the different fits provided by its minimum energy conformer on the GTP template, which include those characteristic of SERT, glycine, GABA, NMDA, kainate and AMPA ligands (see Williams 2018, **Figure 2**) [33]. The wide spectrum of effects on cells produced by low ketamine concentrations, including oxidative stress and apoptosis, is of some concern in the clinical context [34].

The sodium channel blocking effects of antidepressants may also relate to their effects on ATPase activity. Imipramine and fluoxetine decrease rat synaptic plasma membrane N/K ATPase *in vitro* [35]. The neurotransmitter-like interaction of thyroxine with ATP associated enzyme systems has long been of interest. An inverse relationship between thyroxine activation of adenyl cyclase and N/K ATPase activity is observed in cat heart homogenates and rat synaptosomal membranes [36]. Protein kinase phosphorylation of the catalytic alpha unit, containing ATP and ouabain binding sites, modulates ATPase pump activity [37]. The release of the neurotransmitters norepinephrine and 5-HT and their effects on N/K ATPase activity may be subject to reciprocal control [14] [38]. Among other compounds modulating N/K ATPase are long chain fatty acids and glycerol derivatives. Fatty acids have inhibitory and stimulatory effects on purified preparations of Na/K ATPase, depending on the ATP concentration [16]. Under the same experimental conditions, monoacylglycerols activate transport and hydrolytic functions of the enzyme and are antagonised by diacylglycerols. Propafenone, an antiarrhythmic drug, stimulates P-glycoprotein ATPase activity in intact cells and vesicles, a property strongly associated with lipophilicity [39]. In regard to ATP nucleotide template fits, the structures of arachidonic acid, propafenone and T3 relate to their properties of promoting ATPase function. The template fits of the mono- and di-glycerides structures with their distinctive alignment of alkyl chains characterise their stimulatory and inhibitory effects on ATPase function.

Ouabain and digoxin are potent inhibitors of 5-HT uptake by human platelets [40], an observation that concurs with inhibition of the N/K ATPase and the SERT-like fit of ouabain to the GTP template. Ouabain modulates N/K ATPase from the extracellular side, triggering Ca^{2+} release from intracellular stores, whereas digitalis glycosides act intracellularly on ryanodine receptors [41]. The rhamnose and lactone moieties of ouabain are essential for N/K ATPase inhibition, whereas hypertensive properties relate to the steroid nucleus. The functional properties of cGMP and ouabain are inhibited by the ouabain antagonist rostafuroxin, which does not inhibit N/K ATPase [42]. The fitting data in this study depict general template fits of bufalin, rostafuroxin and ouabain involving the steroid nucleus and one specific fit of ouabain involving the sugar linkage. cGMP is natriuretic and molecular modelling demonstrates a potential cGMP docking site in the ouabain-binding pocket of the renal proximal tubule N/K ATPase [15]. cGMP inhibits N/K ATPase in a similar manner to ouabain and both promote phosphorylation. Several models consider the intracellular signaling effects of cardiotonic steroids on N/K ATPase, in terms of reactive oxygen species, reactive nitrogen species and intracellular ionic changes [43]. Intracerebroventricular administration of ouabain to rats provides a model of BPD demonstrating manic and depressive states, decreased N/K ATPase activity, changes in the HPA axis, oxidative stress and the abrogation of such changes by lithium [44]. There is evidence that lithium modulates nucleotide cyclase activity and

second-messenger systems impacting on cell signal transduction [45]. Differences in the nature of sodium and lithium ions mean that Li+ achieves a higher conductance through ion channels and neuronal membranes, attributable to its smaller mass [46]. The benefits of lithium in mouse models of anxiety and seizure are augmented by inhibitors (L-NAME and methylene blue) of NOS and guanylate cyclase [47] [48]. Methylene blue decreases 5-HT uptake by SERT, inhibits ion currents initiated by 5-HT and reduces binding of ASP (used for real-time monitoring of transporter function in single cells) [49]. The potential for interaction between methylene blue and ASP is evident in their their GTP template fits.

Activation of SERT and GPCR are both accompanied by extensive conformational changes within their constituent protein units and the cell membrane. The actions of antidepressants, including ketamine, are characterised by an increase in cAMP and the redistribution of the $G\alpha_s$ protein from lipid rafts to more fluid regions of the cell membrane, and disruption of $G\alpha_s$ /tubulin complexes enabling activation of adenyl cyclase signaling [19] [50]. The competition of receptor agonists, antagonists and antidepressant compounds with radiolabeled [35S]-GTPγ_s for dorsal raphe neuron 5-HT receptors [51] provides supporting evidence for the antidepressant desensitisation of presynaptic 5-HT_{1A} receptors and SSRI therapy. Studies based on crystallography, spectroscopy and computer simulation demonstrate the functional complexity of GPCRs and serve to develop cell-signaling models [52]. In contrast, the relative molecular similarity within ligand structures of SERT and GPCR proteins demonstrates a remarkable simplicity based on the structure of GTP. In regard to the molecular basis of GPCR signaling, receptor ligand and GTP structures contain equivalent core pharmacophores, representing common extracellular and intracellular codes; dimeric receptor messages that may serve as specific activation and transmission mechanisms of $G\alpha$ proteins.

5. Conclusion

In conclusion, the necessary co-operation between SERT and N/K ATPase in achieving 5-HT homeostasis is markedly dependent on attaining correct protein phosphorylation levels. Malfunction of this mechanism is improved by endogenous and synthetic compounds with properties dependent on their molecular similarity to purine nucleotide structure.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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