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RESEARCH PAPER

Steric parameters, molecular modeling and hydropathic interaction analysis of the pharmacology of para-substituted methcathinone analogues

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BACKGROUND AND PURPOSE

There is growing concern over the abuse of certain psychostimulant methcathinone (MCAT) analogues. This study extends an initial quantitative structure–activity relationship (QSAR) investigation that demonstrated important steric considerations of seven 4- (or *para*-)substituted analogues of MCAT. Specifically, the steric character (Taft's steric E_S) of the 4-position substituent affected *in vitro* potency to induce monoamine release via dopamine and 5-HT transporters (DAT and SERT) and *in vivo* modulation of intracranial self-stimulation (ICSS). Here, we have assessed the effects of other steric properties of the 4-position substituents.

EXPERIMENTAL APPROACH

Definitive steric parameters that more explicitly focus on the volume, width and length of the MCAT 4-position substituents were assessed. In addition, homology models of human DAT and human SERT based upon the crystallized *Drosophila* DAT were constructed and docking studies were performed, followed by hydropathic interaction (HINT) analysis of the docking results.

KEY RESULTS

The potency of seven MCAT analogues at DAT was negatively correlated with the volume and maximal width of their 4-position substituents, whereas potency at SERT increased as substituent volume and length increased. SERT/DAT selectivity, as well as abuse-related drug effects in the ICSS procedure, also correlated with the same parameters. Docking solutions offered a means of visualizing these findings.

CONCLUSIONS AND IMPLICATIONS

These results suggest that steric aspects of the 4-position substituents of MCAT analogues are key determinants of their action and selectivity, and that the hydrophobic nature of these substituents is involved in their potency at SERT.

Abbreviations

dDAT, dopamine transporter from *Drosophila*; hDAT, human dopamine transporter; hSERT, human 5-HT transporter; ICSS, intracranial self-stimulation; HINT, hydropathic interactions; MCAT, methcathinone; QSAR, quantitative structure–activity relationship

Tables of Links

TARGETS
DAT, dopamine transporter, SLC6A3
NET, noradrenaline transporter, SLC6A2
SERT, 5-HT transporter, SLC6A4

LIGANDS
Amphetamine
Methamphetamine
Nortriptyline

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013).

Introduction

Synthetic cathinone analogues, specifically N-substituted cathinones such as methcathinone (MCAT) analogues, are new drugs of abuse. These agents are, typically, β-keto analogues of known phenylisopropylamine psychostimulants (e.g. amphetamine, methamphetamine). Thus, cathinone is the β-keto analogue of amphetamine, whereas MCAT is the β-keto counterpart of methamphetamine. As with amphetamine and methamphetamine, MCAT acts primarily at the level of the dopamine transporter (DAT) and functions as a monoamine releaser that selectively increases release of dopamine, in comparison to its lower potency to release 5-HT via its transporter, SERT (Glennon *et al.*, 1987; Cozzi *et al.*, 1999; 2013; Baumann *et al.*, 2012). In behavioural studies, MCAT produces locomotor stimulation in rodents, generalizes to amphetamine and methamphetamine in drug discrimination studies, and produces abuse-related behavioural effects in assays of intracranial self-stimulation (ICSS) and drug self-administration (Kaminski and Griffiths, 1994; Young and Glennon, 1998; Bonano *et al.*, 2013; Cozzi *et al.*, 2013). Because of its methamphetamine-like psychostimulant qualities and high abuse potential, MCAT is now a scheduled (U.S. Schedule I) substance. A number of related MCAT derivatives have now appeared on the clandestine market; their identity and pharmacology have been reviewed (Baumann *et al.*, 2013; Glennon, 2014).

The ability of MCAT and six of its 4- (or *para*-) substituted derivatives to increase neurotransmitter release through DAT and SERT, as well as to produce abuse-related behavioural effects in the ICSS procedure, was recently examined. Abuse-related neurochemical and behavioural effects of these analogues correlated with the steric nature of their 4-position substituents using a general steric parameter (i.e., Taft's steric E_S) (Bonano *et al.*, 2015). Here, we examine this relationship in greater detail by investigating steric parameters that focus on the volume, length and width of the 4-position substituents to gain greater insight as to which steric property might be most relevant for the actions of these agents. Additionally, we constructed homology models of DAT and SERT and docked all seven MCAT analogues in each model in an attempt to explain the experimental quantitative structure–activity relationship (QSAR) findings. The program HINT (Hydropathic INTeractions) (Abraham and Kellogg, 1994) that calculates three-dimensional hydropathy fields was used to analyse the docking interactions.

Methods

QSAR studies

QSAR studies were performed using substituent volume for the 4-position substituent; volume (Vol) was calculated using SYBYL-X 2.1 (Tripos Inc., St. Louis, MO, USA). Verloop's steric parameters examined included substituent length (L), minimum width (B1), and maximum width (B5) (Verloop *et al.*, 1976). Values used for all parameters are shown in Supporting Information Table S1. Linear regressions and statistical analyses were performed between each of these physicochemical parameters and *in vitro* release and *in vivo* ICSS maximum percent baseline facilitation (Bonano *et al.*, 2015) using the software Prism 5.04 (GraphPad, San Diego, CA, USA), and correlations with $P < 0.05$ were considered statistically significant.

Molecular modelling and docking

Homology models of the two human monoamine transporters were built using the 3.0 Å X-ray crystal structure of *Drosophila melanogaster* dopamine transporter (dDAT; PDB entry code 4M48) (Penmatsa *et al.*, 2013) as a template. ClustalX 2.1 (Larkin *et al.*, 2007) was used to align the amino acid sequences of human DAT (hDAT), human SERT (hSERT) and dDAT (see Supporting Information Fig. S1), which were retrieved from the SwissProt-ExPASY databank (accession codes Q01959, P31645 and Q7K4Y6, respectively). Using MODELLER 9.10 (Eswar *et al.*, 2007), a population of 100 models was generated for both hDAT and hSERT whose members possessed varied side chain and, to a lesser extent, backbone conformations. For hDAT, the first 57 residues of the N-terminus, the last 20 residues of the C-terminus and the 12 residues of extracellular loop 2 (for hSERT 76, 13 and 7 residues, respectively) were not modelled due to lack of corresponding residues in the crystal structure template. Because they might influence the structure of the transporters, two sodium ions and a chloride ion were modelled in the binding site using the analogous coordinates from the dDAT crystal structure (see Supporting Information Table S2). Non-structure water molecules were removed. The structures of the compounds to be docked were sketched in SYBYL-X 2.1 and energy-minimized using the Tripos Force Field. The automated docking program GOLDSuite 5.2 (Jones *et al.*, 1997) was employed to dock the *S*-isomer of each substrate into each of the 100 hDAT and hSERT models using the GoldScore

fitness function. The transporter binding cavity was defined to include all atoms within a 5 Å radius from the co-crystallized nortriptyline molecule in the dDAT homologue. Docking simulations were run without constraints and were not subjected to early termination. A total of 10 genetic algorithm docking runs were performed for each docked structure. Using clustering techniques and in-house SYBYL Programming Language (SPL) scripts, putative modes of interaction were obtained from the population of human transporter models for all docked substrates. Common favourable transporter–substrate complexes, at hDAT and hSERT, were identified for all substrates based upon highly populated clusters, favourable GoldScores and stereoelectronic complementarity with the binding site. Finally, the transporter–substrate complexes were energy-minimized in SYBYL-X 2.1.

The HINT (Kellogg and Abraham, 2000) facility within SYBYL 8.1 (Tripos Inc.) was used to quantify the nature and magnitude of the molecular interactions between the 4-position substituent of the MCAT analogues and the hDAT and hSERT models. For the initial partitioning phase, the dictionary method was used for the protein and the calculation method was used for the ligands. The 'proton suppress' option was disabled in the Intermolecular HintTable calculation phase. All other parameters were set to their default values. Atom-based interactions involving atoms of the 4-position substituent were extracted from the resulting Hint table and tabulated.

Results

Correlational studies

The neurochemical and behavioural data employed in the QSAR analysis are shown in Table 1, and the results of the correlational studies are shown in Table 2. The values of log EC_{50} to increase *in vitro* monoamine release via DAT was negatively correlated with increasing volume (R = –0.803) and maximal substituent width (R = –0.807), whereas log EC_{50} at SERT was positively correlated with increasing volume (R = 0.825) and length (R = 0.903) of the 4-position substituent. Selectivity for DAT versus SERT was correlated with volume (R = –0.972; P = 0.0002) and maximal width (B5) (R = –0.917; P = 0.0039) (Figure 1A and B respectively).

Maximal ICSS facilitation was negatively correlated with all four steric parameters: volume (R = –0.915), length (R = –0.773), minimum width (R = –0.778) and maximum width (R = –0.814) (Table 2). It might be noted that internal correlations were found between volume and both substituent length (R = 0.814; P = 0.0258) and maximal width (R = 0.935; P = 0.00020), as well as between length and maximal width (R = 0.798; P = 0.0315); the relationship between volume and maximal width (B5) is shown in Figure 1C.

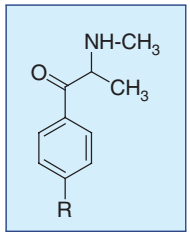
Molecular modelling

dDAT shares >50% sequence identity with its mammalian counterparts and possesses a pharmacological profile that is a hybrid of mammalian DATs, SERTs and noradrenaline transporters (NETs) (Penmatsa *et al.*, 2013), and hDAT and hSERT have a greater than 90% sequence identity with their rat orthologues (see Supporting Information Figs S2 and S3).

The homology model of hDAT (Figure 2) is consistent with the available site-directed mutagenesis data. The putative MCAT binding site includes 12 amino acid residues; the cognate residues in the dDAT crystal structure also interact with the co-crystallized nortriptyline inhibitor. Of these, three are different in hDAT versus hSERT (hDAT: F76, S149 and V152; hSERT: Y96, A169 and I172). For hDAT, the docked MCAT solutions show extensive spatial overlap in the

Table 1

In vitro release potency and *in vivo* behavioural ICSS effects of MCAT analogues



Agent	Abbreviation	R	DAT EC_{50} (nM)[a]	SERT EC_{50} (nM)[a]	DAT selectivity[b]	ICSS maximum % baseline Facilitation
Methcathinone	MCAT	–H	12.5	3860	309	191.9
Flephedrone	4-F MCAT	–F	83.4	1290	15.4	156.3
Methedrone	4-OCH_3 MCAT	–OCH_3	506	120	0.24	110.9
4-Chloromethcathinone	4-Cl MCAT	–Cl	42.2	144	3.40	114.9
4-Bromomethcathinone	4-Br MCAT	–Br	59.4	60	1.01	118.0
Mephedrone	4-CH_3 MCAT	–CH_3	49.1	118	2.41	102.5
4-Trifluoromethylmethcathinone	4-CF_3 MCAT	–CF_3	2,700	190	0.07	90.9

[a]EC_{50} values and ICSS data are from Bonano *et al.* (2015). [b]DAT selectivity calculated as SERT EC_{50} ÷ DAT EC_{50}.

Table 2

Results of correlational analysis between steric parameters, *in vitro* potencies at DAT and SERT, and maximal ICSS facilitation

Physicochemical parameter	log EC_{50} DAT	log EC_{50} SERT	log DAT selectivity	Maximal ICSS facilitation
Volume (Vol)	**$R = -0.803$**	**$R = 0.825$**	**$R = -0.972$**	**$R = -0.915$**
	$P = 0.03$	**$P = 0.02$**	**$P < 0.01$**	**$P < 0.01$**
Length (L)	$R = -0.404$	**$R = 0.903$**	**$R = -0.840$**	**$R = -0.773$**
	$P = 0.26$	**$P < 0.01$**	**$P = 0.03$**	**$P = 0.04$**
Minimum width (B1)	$R = -0.416$	$R = 0.762$	$R = -0.727$	**$R = -0.778$**
	$P = 0.28$	$P = 0.05$	$P = 0.06$	**$P = 0.04$**
Maximum width (B5)	**$R = -0.807$**	$R = 0.720$	**$R = -0.917$**	**$R = -0.814$**
	$P = 0.03$	$P = 0.07$	**$P < 0.01$**	**$P = 0.03$**

Significant correlations ($P < 0.05$) are highlighted in bold.

putative binding mode (Figure 2A). The models suggest that the charged terminal amine of the MCAT analogues interacts favourably with the D79 side chain carboxylate group, as has been reported for dopamine (Huang and Zhan, 2007; Indarte *et al.*, 2008) and releasing agents such as *S*(+)amphetamine (Indarte *et al.*, 2008). Previous mutations of this residue (i.e. D79A, D79G, D79E) suggest that it plays a role in the binding of dopamine and these mutations led to the loss of the dopamine reuptake function of the transporter (Kitayama *et al.*, 1992). In addition, our model shows that a hydrogen bond exists between the backbone carbonyl group of F76 and the charged terminal amine. It has already been shown that the mutation F76A dramatically reduced the V_{max} for dopamine uptake (Lin *et al.*, 1999).

Other residues, including F76, V152, Y156, S422 and G426, engage in hydrophobic interactions with the phenyl ring. Earlier studies of DAT indicated that V152, F156 and S422 play a crucial role in the transport of dopamine. Mutants of hDAT (V152I, V152A and V152M) displayed remarkably reduced dopamine uptake (Lee *et al.*, 2000). Mutations of the strictly conserved residue Y156 (Y156A and Y156C) resulted in inactive transporters (Beuming *et al.*, 2008). Also, S422 has been implicated to bind dopamine through an interaction with its hydroxyl groups; a reported mutation of this residue (S422A) resulted in decreased dopamine uptake (Beuming *et al.*, 2008).

Our model predicts that F76, F320 and F326 interact with the α-methyl group. The N-methyl group is directed towards a small pocket defined by F76, A77, F320, S321 and L322. The 4-position substituent is directed towards S149. Recent studies have indicated that, in hDAT, S149 might be important for coordination of the 4-OH group of dopamine (Koldsø *et al.*, 2013). No specific interactions with the carbonyl oxygen atom of MCAT analogues were identified in the binding site.

All the compounds were orientated in a similar manner in the hSERT binding pocket (Figure 2B). The key interactions observed in each of the seven compounds were an ionic interaction of the charged amine with the side chain carboxylate group of D98. This residue has been predicted to form the same interaction with the positively charged side chain of 5-HT in a previously published model (Koldsø *et al.*, 2013). D98G, D98A and D98N mutants were found to be inactive in uptake of 5-HT; only the D98E mutation rendered the transporter active, which implies that the ionic interaction with D98 is important (Celik *et al.*, 2008; Andersen *et al.*, 2010).

Another observed interaction is a hydrogen bond between the backbone carbonyl of Y95 and the charged amine. Mutation studies of these residues Y95E and Y95R rendered the transporter non-functional in a 5-HT uptake assay, whereas Y95F, Y95Q and Y95E decreased transporter activity as compared to the wild type (Andersen *et al.*, 2010).

The aromatic ring is buried in a hydrophobic pocket bound by Y95, I172, Y176, F341, S438 and G442. In addition, our model predicts a hydrophobic interaction with the aromatic ring of Y95 and G338 with the N-methyl group, and F341 with the α-methyl side chain. A pocket of hydrophobic residues lined by A169, I172, A173, T439 and L443 surrounds the 4-position substituent. Residues (Y95, I172, Y176, F341, S438, G442, A169, I172, T439 and L443) that make hydrophobic interactions in our docking studies have been mutated previously, and the mutants showed decreased transporter activity in 5-HT uptake assays as compared to the wild type (Andersen *et al.*, 2010).

All in all, the graphics models of hDAT and hSERT seem consistent with the available mutagenesis data and can be viewed as reliable models for our docking studies.

HINT is a computational tool that calculates three-dimensional hydropathic (primarily hydrophobic and polar) interactions between a molecule and its protein target (Abraham and Kellogg, 1994). HINT calculations were performed on the energy-minimized hDAT and hSERT transporter–ligand complexes, and the total HINT scores, as well as hydrophobic and polar interaction contributions, are shown (reported only for 4-position substituent interactions) in Table 3.

For hDAT, total and polar contributions were found to be mostly negative (Table 3). Although the hydrophobic contribution was positive, no significant correlation was identified between potency and the total HINT score, nor for the polar or hydrophobic contribution, of the 4-substituent (Table 4).

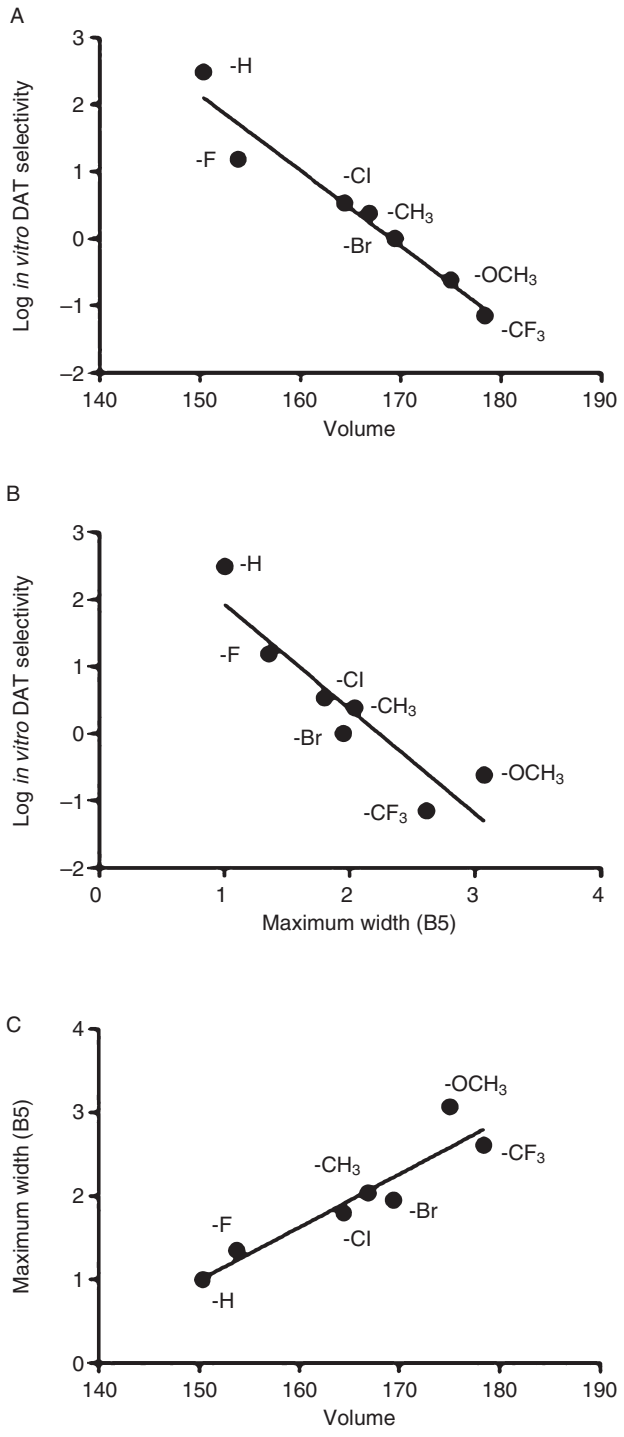


Figure 1

Relationship between (A) *in vitro* selectivity for DAT-versus-SERT-mediated monoamine release and the volume of the 4-position substituent (R = –0.972; P = 0.0002). (B) *In vitro* selectivity for DAT-versus-SERT-mediated monoamine release and maximal width (B5) (R = –0.917; P = 0.0039) and (C) between the volume of the 4-position substituent and maximal width (B5) (R = 0.935; P = 0.0020).

At hSERT, the total HINT scores were positive (Table 3). Even though the polar contribution was negative, this was offset by a higher positive value from the hydrophobic contribution. There was a significant correlation between potency and total and polar HINT scores (R = –0.864, P = 0.012; R = 0.841, P = 0.018, respectively), as well as with the hydrophobic contribution (R = –0.910; P = 0.004) (Table 4; Figure 3).

Discussion and conclusions

Simply stated, there are at least three major factors that seem to be involved in understanding structural variations within a set of compounds that produce a common response in a bioassay test system (Hansch and Fujita, 1995): electronic, hydrophobic and steric. One of the first steric parameters to be employed was the Taft steric E_S (Taft, 1952; 1953). This parameter was derived by measuring rates of hydrolysis of certain esters, and it is a composite reflecting the contribution of steric strain and steric hindrance of motion (Taft, 1953). Some authors have suggested involvement of polar or hyperconjugative effects (see MacPhee *et al.*, 1978) in the E_S parameter, and several modified E_S parameters were developed (e.g. see Karelson, 2000). The E_S parameters are often useful for preliminary QSAR analyses because they integrate multiple factors and provide a global view of steric attributes. Unlike most of these steric parameters that are experimentally derived, Verloop *et al.* (1976) devised several computationally derived parameters to define steric constraints of substituent groups: L (substituent length), B1 (minimal substituent width) and B5 (maximal substituent width). Substituent volume is another computationally derived parameter that can be used to define substituent groups. Hence, the Verloop and volume parameters permit deconstruction of steric bulk into subcomponents that might contribute differently to structure–activity relationships. Accordingly, in the event that steric factors are implicated by an initial QSAR analysis using E_S, subsequent analysis with computationally derived parameters permits a more refined evaluation of steric factors that might generate added precision. Some have even argued that Verloop parameters offer a significant improvement over other parameters in the ability to offer mechanistic insight (Harper *et al.*, 2012).

Undoubtedly, multiple structural features (e.g. the terminal amine, the aromatic ring) are involved in the actions of MCAT analogues (see the Molecular modelling section), but these structural features are common to the structural scaffold of all the analogues examined here. That is, the current MCAT analogues vary only with respect to the nature of their 4-position substituent. Consequently, this substituent was the focus of this investigation. We have previously demonstrated that the actions of seven MCAT analogues on dopamine release, 5-HT release and ICSS effects are significantly correlated with the Taft steric E_S of their 4-position substituent (Bonano *et al.*, 2015). The present findings were meant to extend this analysis and they support the earlier conclusion that steric effects, as initially identified by Taft's steric E_S, of the 4-position substituents, play a major role in the action of MCAT analogues. That is, as the size of the 4-position substituent increases, potency at DAT

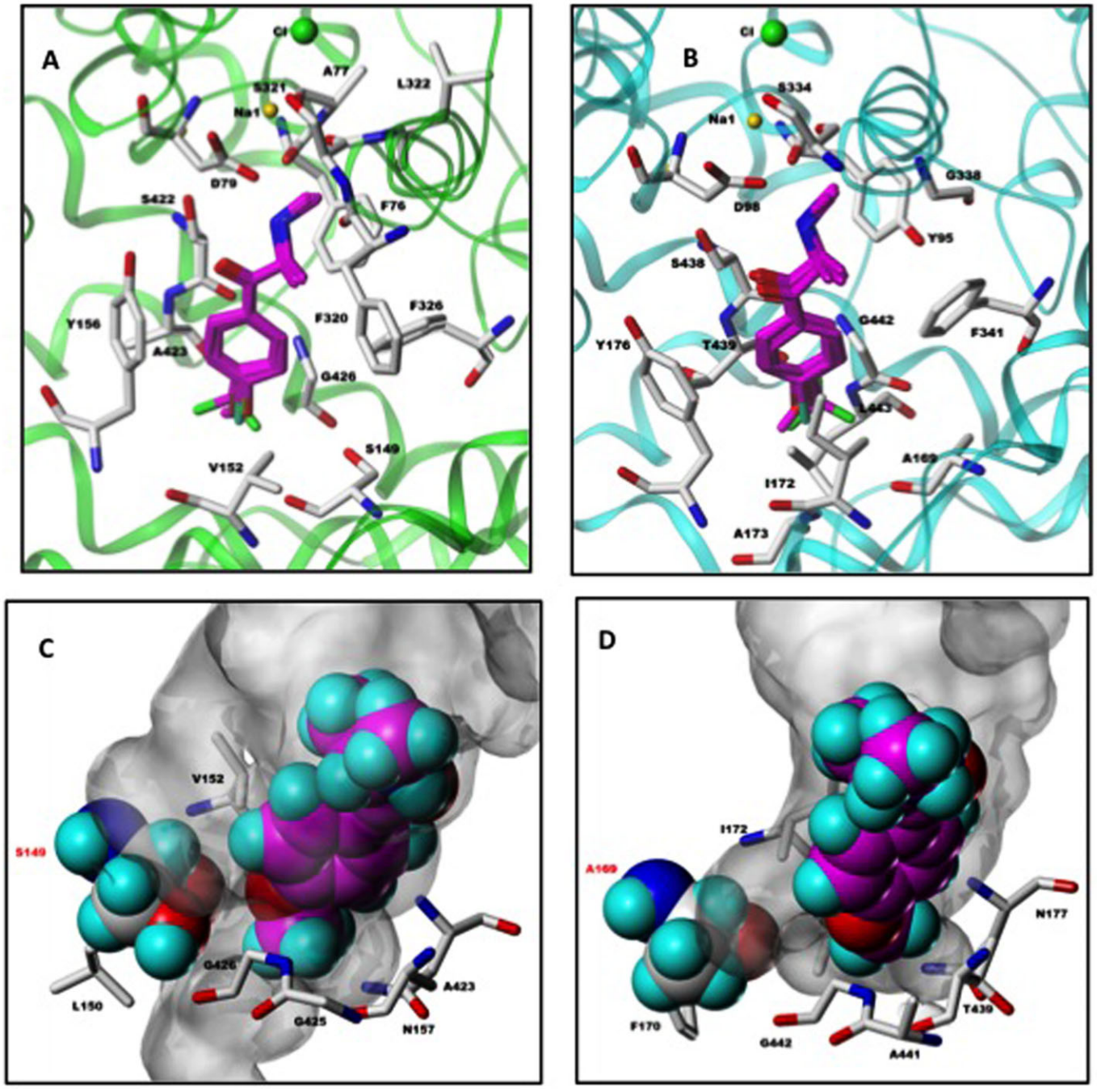


Figure 2

Superimposed poses of all seven MCAT analogues in the putative binding site of hDAT (A) and hSERT (B). Interacting amino acids within 5 Å are indicated. Unfavourable interactions of hDAT S149 with the 4-position substituents (shown for the 4-methoxy analogue; C) that are not seen in hSERT due to the smaller side chain of A169 (again, shown for the 4-methoxy analogue; D). The Connolly surface (shown in grey in C and D) represents the channels in both transporters.

decreases whereas potency at SERT increases. This seemingly reciprocal relationship is echoed by DAT-versus-SERT selectivity.

Due to intercorrelations between some of the parameters examined for the current data set (e.g. between Vol and B5), it was not possible to identify a single 'specific' steric parameter as being the most important. Nevertheless, the higher correlation coefficients (and consistent identification) of the Vol parameter suggest that it is the total volume of the 4-position substituent that is likely to be most important. Because the maximal widths (i.e. B5) of the 4-position substituents varied over a relatively narrow range, additional compounds will need to be (and are being) prepared and examined to further address this issue.

Table 3

Calculated HINT scores for interaction of MCAT analogues with hDAT and hSERT

Agent	HINT score at hDAT (4-substituent only) Total	Polar	Hydrophobic
MCAT	0	0	0
4-F MCAT	22	–11	33
4-OCH_3 MCAT	–88	–231	143
4-CH_3 MCAT	–23	–154	131
4-Cl MCAT	2	–132	134
4-Br MCAT	–13	–173	160
4-CF_3 MCAT	–5	–63	58
	HINT score at hSERT (4-substituent only)		
MCAT	0	0	0
4-F MCAT	51	–12	63
4-OCH_3 MCAT	125	–154	279
4-Cl MCAT	136	–102	238
4-Br MCAT	135	–157	292
4-CH_3 MCAT	78	–173	251
4-CF_3 MCAT	97	–67	164

Table 4

Correlations between HINT scores and log EC_{50} values at hDAT and hSERT

HINT score	log EC_{50} DAT	log EC_{50} SERT
Total	R = 0.044	***R* = –0.864**
	P = 0.93	***P* = 0.01**
Polar contribution	R = 0.103	***R* = 0.841**
	P = 0.84	***P* = 0.02**
Hydrophobic contribution	R = 0.057	***R* = –0.910**
	P = 0.90	***P* < 0.01**

Statistically significant correlations ($P < 0.05$) are highlighted in bold.

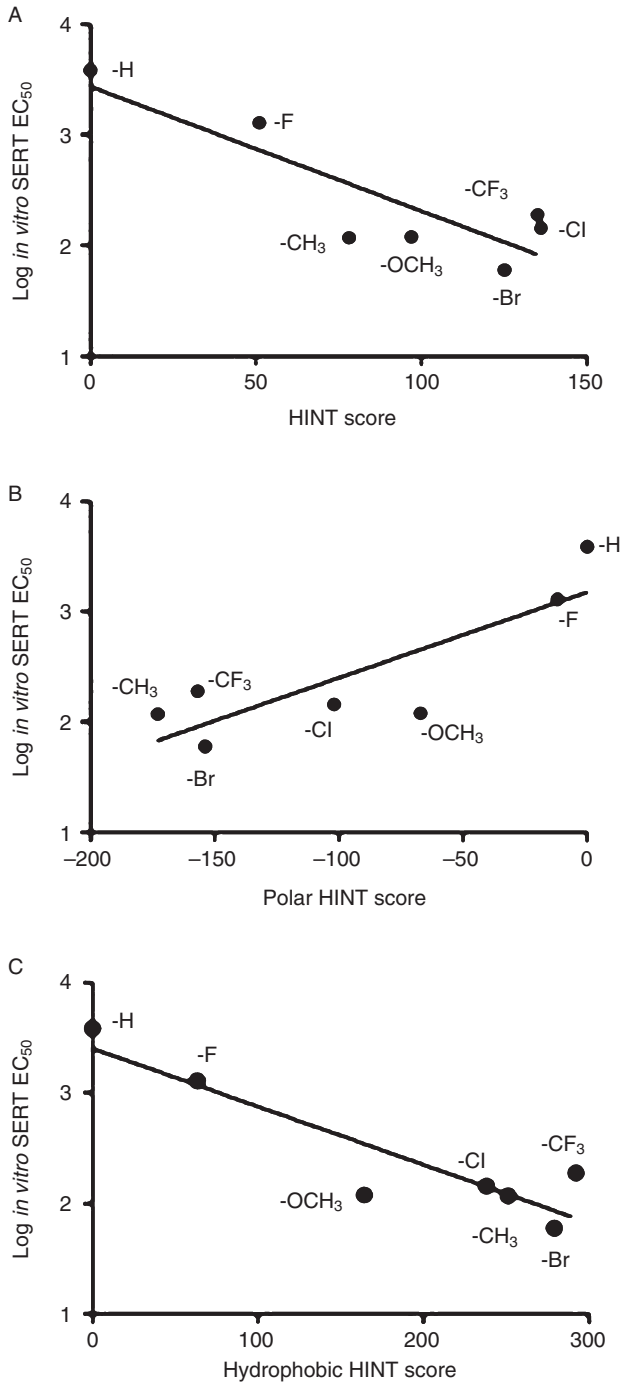


Figure 3

Relationship between SERT *in vitro* potency (log EC_{50}) and (A) total HINT score, (B) polar HINT score and (C) hydrophobic HINT score.

Our modelling studies resulted in new homology models for hDAT and hSERT. Although loss-of-function results from studies using transporter mutants cannot be taken as definitive evidence for involvement of specific ligand–amino acid interactions, the present findings are not inconsistent with the available mutagenesis data.

Docking studies showed that large substituents at the MCAT 4-position are better tolerated by hSERT than by hDAT. That is, the presence of an S149 residue in hDAT instead of A169 in hSERT makes the binding pocket of hDAT less accessible to compounds with large 4-position substituents. This could explain the DAT-versus-SERT selectivity data shown in Table 1. The results are consistent with the QSAR studies described earlier.

The results of both the QSAR investigation and the docking studies support the concept that the potency of the MCAT analogues is correlated with certain features of the 4-position substituent: DAT does not accommodate the larger

substituents whereas SERT does. However, the results failed to explain why agents with larger 4-position substituents displayed increased potency at SERT. The results of the HINT analysis indicated that hydrophobic interactions by the 4-position substituents are important contributors to potency. Taken together, these results suggest that hydrophobic interactions provided by the 4-position substituent are necessary for potency at hSERT, whereas polar interactions (particularly unfavourable ones) at the 4-position might play a role in determining potency at hDAT (although the latter findings were not statistically significant). (To put this in perspective, numerous studies have shown that a HINT score of 500 units is equivalent to approximately 1 kcal·mol^{-1}.) This conclusion is consistent with a smaller and more polar (due to the presence of the serine, S149, residue) binding pocket in hDAT that cannot readily accommodate the 4-position substituents of MCAT analogues, and a larger, less polar binding pocket associated with an alanine (A169) residue in hSERT.

Interestingly, once we had completed our homology modelling and docking studies, we became aware of a study (Severinsen *et al.*, 2012) where a series of 1-phenylpiperazine analogues was docked at DAT and SERT homology models, construced using the bacterial leucine transporter (LeuT) as template. Although the templates and the agents used in docking were quite different, both studies showed the aromatic rings located in similar binding pockets (Supporting Information Table S3) and that aryl 4-position substituents can play a role in selectivity.

The overall conclusions of this QSAR and modelling/docking investigation are that (i) MCAT analogues with small 4-position substituents (as measured, e.g. by substituent volume) favour binding at DAT; (ii) larger substituents favour binding at SERT; and that (iii) the hydrophobic nature of these substituents modulates potency at SERT. This provides some of the first insight into the varying actions and mechanisms of action of synthetic cathinone analogues that have been demonstrated to be quite disparate in this regard (Glennon, 2014).

Acknowledgements

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Author contributions

R. A. G. designed the current investigation. J. S. B., under the direction of S. S. N. and M. L. B., provided the ICSS data and, together with R. A. G. identified the initial correlations with Taft's steric E_S. F. S. conducted the present QSAR studies, constructed the hSERT model and conducted the hSERT docking studies, whereas R. K. constructed the hDAT model and conducted the hDAT docking studies. P. D. M. assisted in the docking studies, wrote the required SYBYL Programming Language scripts and conducted the HINT analysis. J. S. P. and M. H. B. provided the *in vitro* neurochemical data. R. A. G. prepared the initial draft of the manuscript, with experimental contributions and proofreading by all other co-authors.

Conflict of interest

The authors report no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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Figure S1 ClustalX-based alignment of dDAT crystal structure, hDAT (UniProt ID = Q01959) and hSERT (UniProt ID = P31645) primary amino acid sequences.
Figure S2 Sequence alignment of hDAT and rDAT (UniProt ID: Q01959 and P23977 respectively). Residues colored in green are predicted to contribute to the substrate binding site.
Figure S3 Sequence alignment of hSERT and rSERT (UniProt ID: P31645 and P31652 respectively). Residues colored in green are predicted to contribute to the substrate binding site.
Table S1 Parameters and values used in the QSAR analysis.
Table S2 Residues interacting with the two sodium ions and the chloride ion in dDAT, hDAT, and hSERT.
Table S3 Amino acid residues implicated from docking studies.