Supplementary Information

Identification of Histamine H_3 Receptor Ligands Using a New Crystal Structure Fragment-based Method

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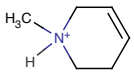
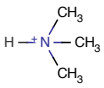
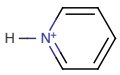
Supplementary Table 1: The fragments used to build the H_3 pharmacophore. The selected representative fragments for H_3 yielded 9 pharmacophore elements and a tenth, for 5.46x461, was added based on docking of reference ligand structures in a H_3 structure model. The aromatic pharmacophore elements are based on multiple fragments and were manually placed in their midst. The pharmacophore element types are abbreviated; Ar: aromatic, HBA: hydrogen bond acceptor, HBD: hydrogen bond donor, HYD: hydrophobic and ICT: ionic cation. *Residue positions are indexed using the GPCRdb scheme for generic residue numbering[1], which is the Ballesteros-Weinstein scheme[2] adjusted for helical bulges and constrictions observed in crystal structures.

Residue Position*	GPCR	PDB ID	Ligand Moiety	Element (Fig. 1)	Element Type	Size (Å) / Tolerance
W3.28	ADRB1	2Y00, 4AMJ		R9	Ar	1.5
D3.32	5-HT1B	4IAR		D1	HBD	1.0
				P4	ICT	0.75
	ADRB1	2Y03		D2	HBD	1.0
				P5	ICT	0.75
Y3.33	M3	4DAJ		R8	Ar / HYD	1.5
	M2	3UON				
	H1	3RZE				
Y6.51	ADRB1	2Y00, 2Y03, 2T04				
E5.46x461	H3	-		P6	ICT	0.75
				D3	HBD	1.0
				R10	Ar	1.5
W6.48	H1	3RZE		R7	Ar	1.5

Supplementary Table 2: Additional results from the IP-one assay

Compound	pIC_{50}±SEM	IC_{50} (μM)	n
21	4.68±0.10	20.9	5
42	4.38±0.11	42.1	5
64	3.58±0.17	261	4
68	4.66±0.20	22.0	2
75	3.92±0.18	121	3

Supplementary Table 3: Additional results from the radioligand competition binding assay

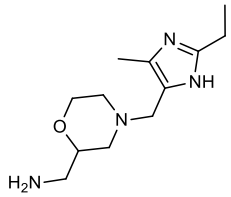
Compound	pKi±SEM	Ki (μM)	n
9[c]	5.76±0.25	1.75	3
20[c]	5.37±0.12	4.24	3
21[a]	-	>50.0	3
42[b]	4.83±0.08	14.8	4
44[b]	5.55±0.06	2.82	3
64	4.06±0.11	86.7	3
67	5.45±0.19	3.52	3
68[a]	-	>300	3
75	5.32±0.04	4.82	3

a Compound had little to no effect on radiolabeled ligand binding in the concentrations examined.
b Compound unable to outcompete radiolabeled ligand to NSB levels (non-competitive interaction).
c A continued decrease in potency observed upon repeated experimentation, possibly due to compound breakdown.
Ki values obtained by the method of Cheng-Prusoff using a Kd value of 0.15 nM for [3H]N-α-methylhistamine.

Supplementary Table 4: Filtering criteria for the eMolecule compounds

Parameter	Range
Molecular weight	200 - 500
Rotatable bonds	2 - 10
Rings	≤ 5
Aromatic carbons	≥ 6
Aliphatic rings	≤ 2
State penalty (Epik)	≤ 2
Reactive groups (#rtvFG)	0
Polar surface area (PSA)	< 150
Hydrophilic component of the solvent accessible surface area (FISA)	7 - 330
Predicted solubility (PlogS)	-6 - 0.5
Predicted logP (PlogPo/w)	< 5
Hydrogen bond acceptors	1 - 10
Hydrogen bond donors	1 - 5
Charged donor groups	≥ 1
Charged amines	1 - 3
Neutral amines	≤ 1
Total charge	2
Number of negative atoms	0

Supplementary Chart 1: Structures of the purchased pharmacophore hits (1-44) and primary assaying hit analogs (45-76). The 2D structures were drawn in ChemBioDraw v. 14.0.



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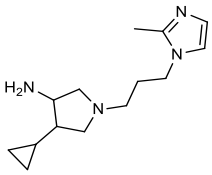

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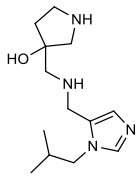

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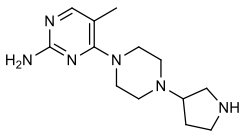

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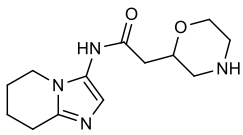

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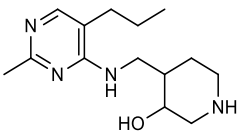

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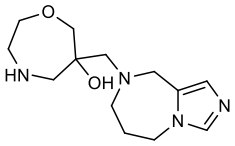

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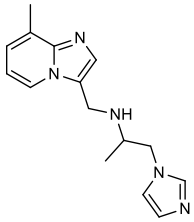

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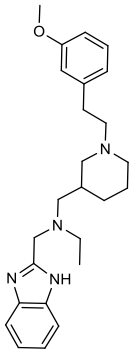

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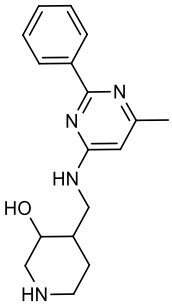

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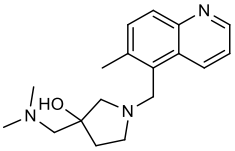

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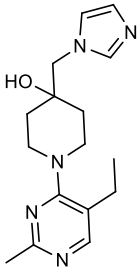

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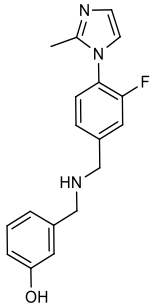

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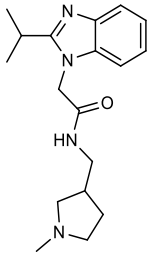

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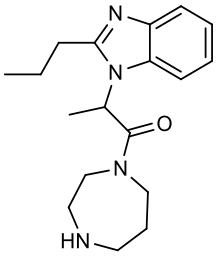

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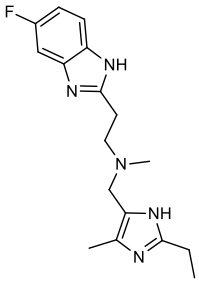

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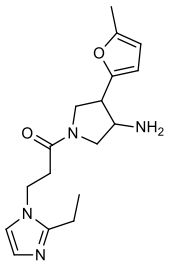

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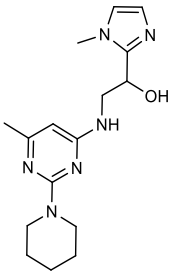

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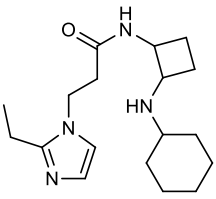

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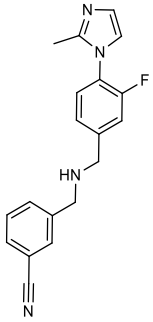

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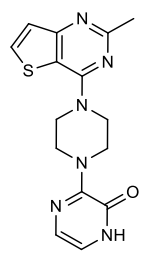

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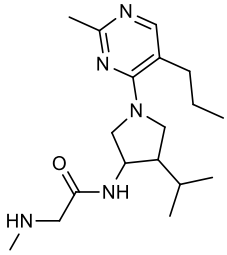

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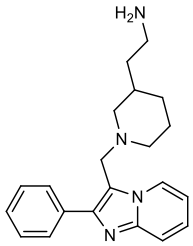

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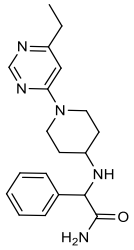

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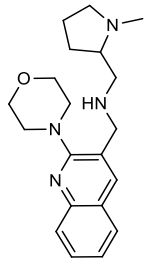

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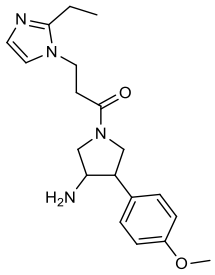

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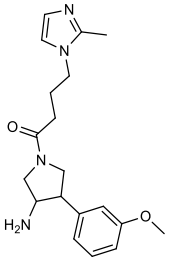

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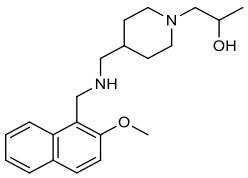

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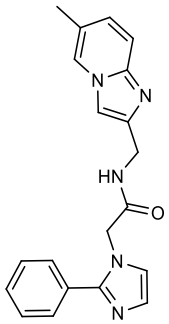

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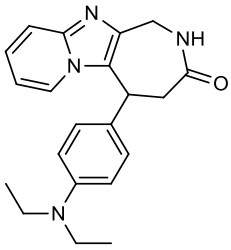

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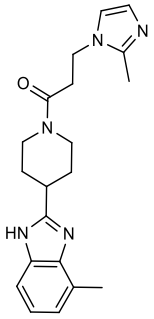

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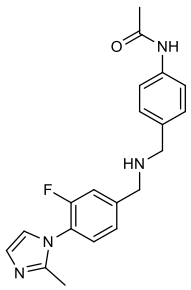

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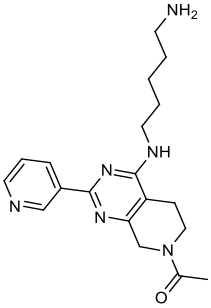

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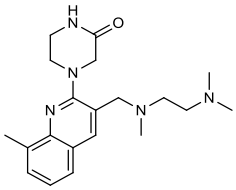

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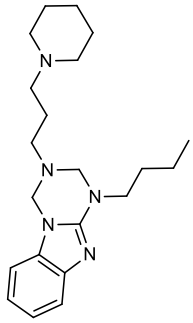

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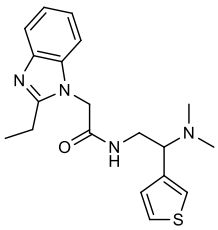

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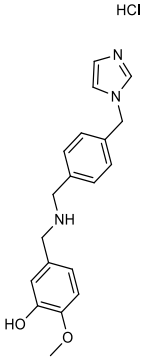

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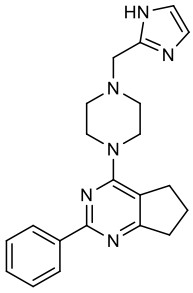

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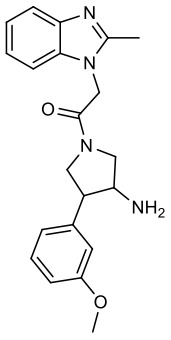

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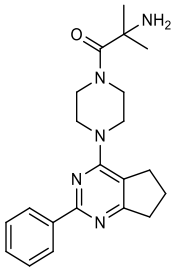

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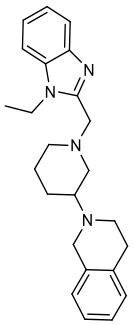

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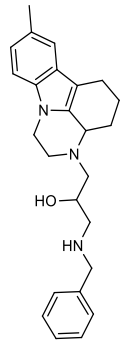

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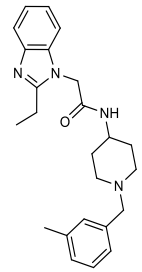

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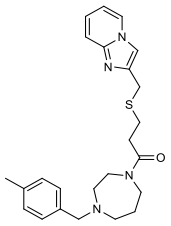

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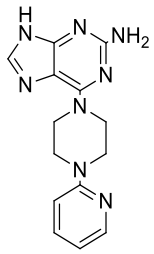

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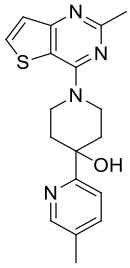

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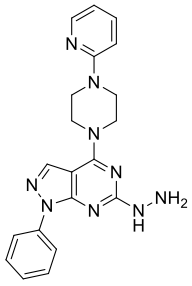

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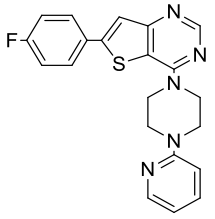

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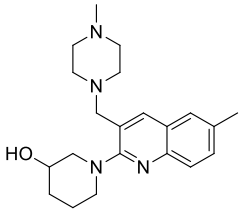

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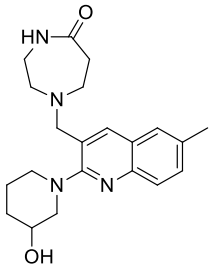

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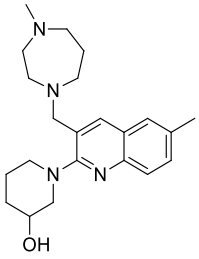

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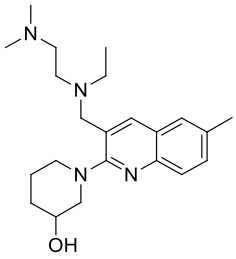

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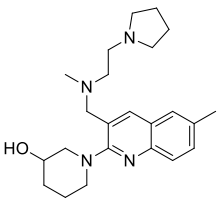

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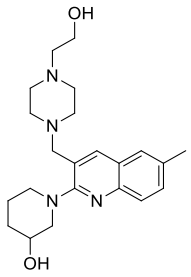

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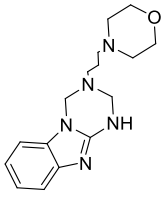

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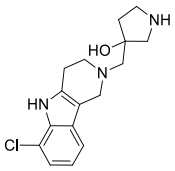

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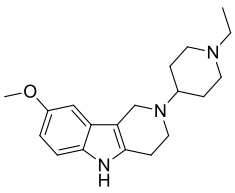

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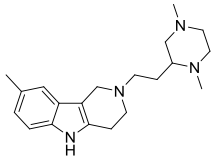

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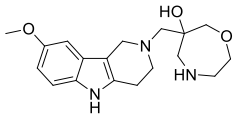

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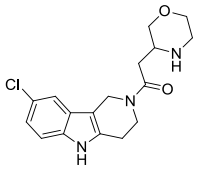

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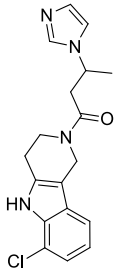

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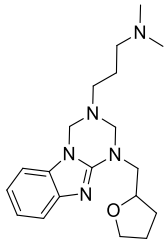

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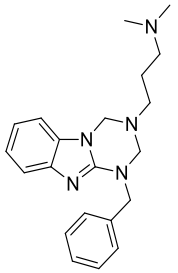

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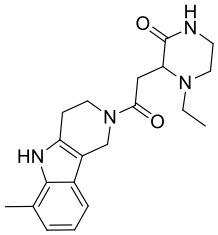

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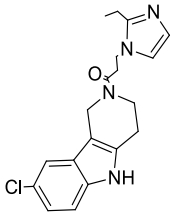

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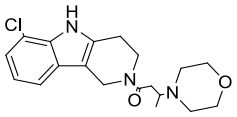

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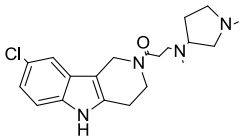

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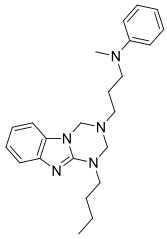

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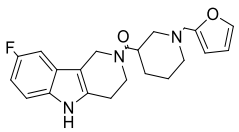

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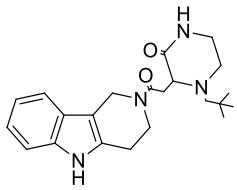

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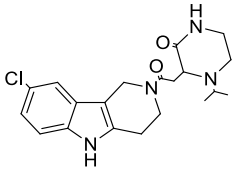

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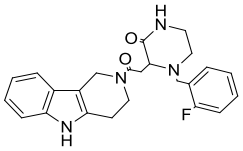

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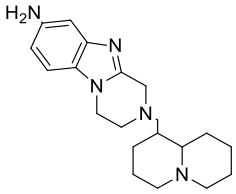

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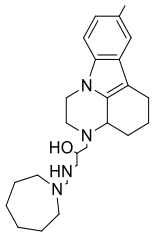

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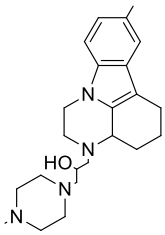

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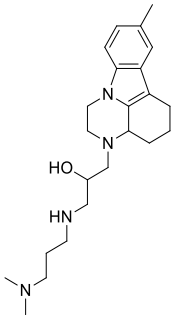

76
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