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1-30 November 2020

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Synthesis and Biological Evaluation of Novel Long-Chain Arylpiperazine Derivatives Targeting Multiple Serotonin Receptors as Potential Drugs for Autism Spectrum Disorder

Enza Lacivita [1], Mauro Niso [1], Margherita Mastromarino [1], Andrea G. Silva [2], Cibell Resch [3], Andre Zeug [3], M. Isabel Loza [2], Marián Castro [2], Evgeni Ponimaskin [3], Marcello Leopoldo [1,*]

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[3] Cellular Neurophysiology, Hannover Medical School, Hannover, Germany

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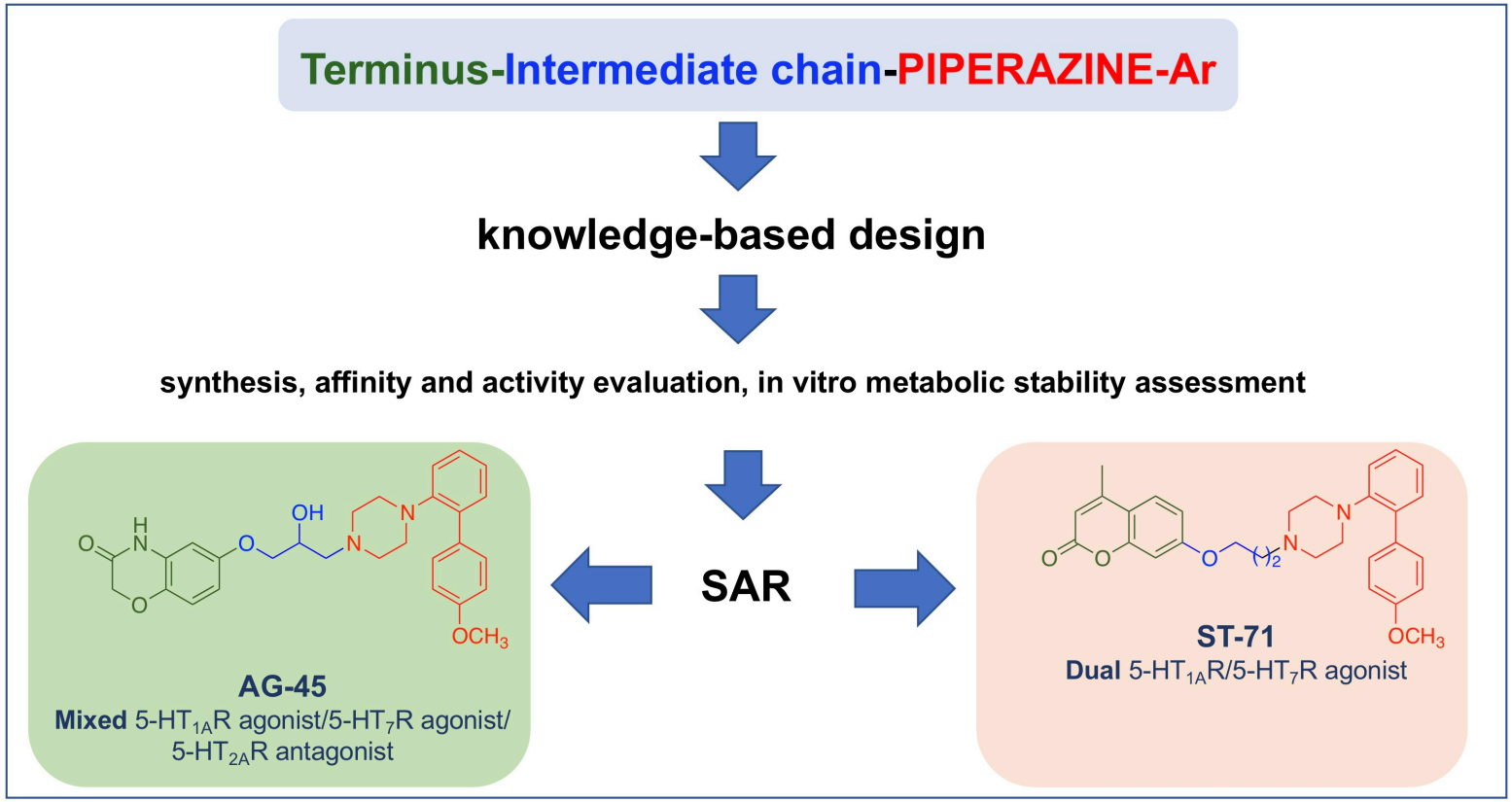
UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Synthesis and Biological Evaluation of Novel Long-Chain Arylpiperazine Derivatives Targeting Multiple Serotonin Receptors as Potential Drugs for Autism Spectrum Disorder

Graphical Abstract



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Abstract

Multiple pieces of evidence suggest that targeting serotonin receptors might have the potential to treat the core symptoms of autism spectrum disorder. We have pursued a knowledge-based design strategy to identify novel arylpiperazine derivatives with dual serotonin 5-HT_{1A}/5-HT_7 receptor agonist or mixed serotonin 5-HT_{1A} agonist/5-HT_7 agonist/5-HT_{2A} receptor antagonist properties. Seventeen new compounds were synthesized and tested in radioligand binding assay at serotonin 5-HT_{1A}, 5-HT_7, and 5-HT_{2A} receptors, which are predicted to improve core symptoms of ASD. We identified a dual 5-HT_{1A}R/5-HT_7 receptor agonist and a mixed 5-HT_{1A} agonist/ 5-HT_7 agonist/5-HT_{2A} receptor antagonist. Both compounds are metabolically stable in vitro and have suitable central nervous system drug-like properties.

Keywords: autism; serotonin; arylpiperazine; SAR

Introduction

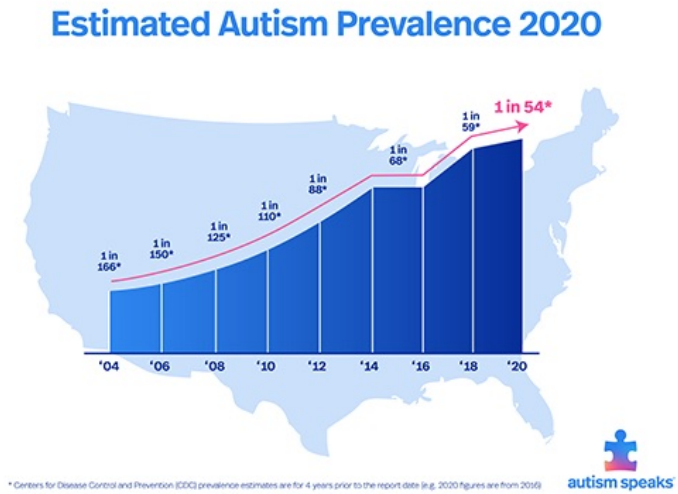
Autism Spectrum Disorder (ASD) refers to a group of neurodevelopmental disorder characterized by:

- Persistent deficits in social communication and social interaction across multiple contexts
- Restricted, repetitive patterns of behavior, interests, or activities

(as defined by the Diagnostic and Statistical Manual of Mental Disorders DSM–5)

The frequency of ASD is increasing, with present rates of about 1 in 100 children in Europe and 1 in 54 in the United States

(www.cdc.gov/ncbddd/autism/data.html)



Etiological Factors

GENETIC SUSCEPTIBILITY

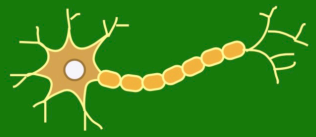
Short nucleotide polymorphisms
Copy number variants
De novo mutations
Epigenetic modifications

ENVIRONMENTAL RISK

Perinatal infections
Maternal pharmacological exposures
Obstetric complications
Early life stress
Advanced parental age

Neuropathologies

ABNORMAL BRAIN DEVELOPMENT & FUNCTION



Altered synaptic transmission
Purkinje cell deficits
Altered neurochemical signaling
Excitatory/inhibitory imbalance
Disrupted circuit connectivity

Behavioral abnormalities

Comorbidities

GUT DYSFUNCTION

Compromised intestinal barrier
Abnormal gut motility
Abdominal pain
Acid reflux
Enteric inflammation

MICROBIAL DYSBIOSIS

Altered bacteria abundance
Altered bacteria diversity
Changes in gene expression
Changes in metabolite profile

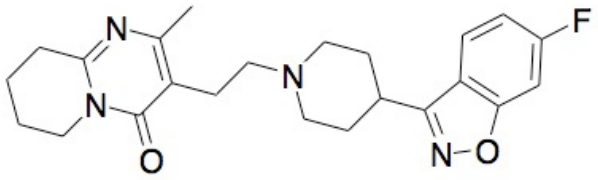
IMMUNE DYSREGULATION

Microglial activation
Altered T cell distributions
Altered brain & blood cytokines
Altered immunoglobulin levels
Abnormal monocyte and NK cell responses

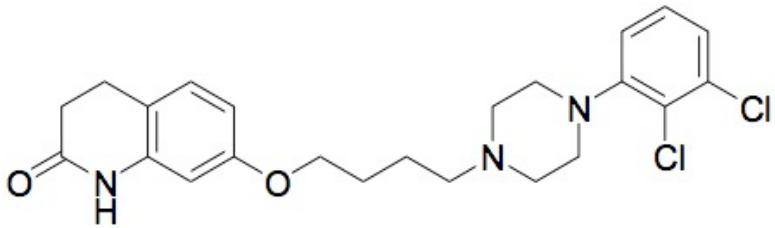
(adapted from Vuong & Hsiao Biol. Psychiatry 2017)

Current psychotropic drugs do not treat core symptoms of ASD

FDA approved



Risperidone

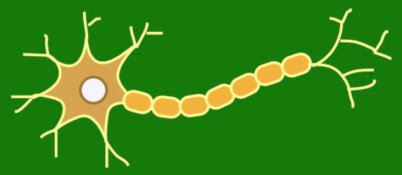


Aripiprazole

These medicines treat irritability associated with the ASD. By relieving irritability they often improve sociability while reducing tantrums, aggressive outbursts and self-injurious behaviors.

Drug targets for ASD neuropathologies

ABNORMAL BRAIN DEVELOPMENT & FUNCTION



Altered synaptic transmission

Purkinje cell deficits

Altered neurochemical signaling

Excitatory/inhibitory imbalance

Disrupted circuit connectivity

- PI3K/mTOR pathway
- Insulin-like Growth Factor-1
- SHANK proteins
- ROCK kinases

- Serotonin system
- Cholinergic system
- Oxytocin system
- Vasopressin System

- $mGlu_5$ receptor
- group II mGlu receptors
- Glycogen synthase kinase 3 (GSK-3)
- NMDA receptors
- GABA receptors

For details see: Lacivita E, Perrone R, Margari L, Leopoldo M. Targets for Drug Therapy for Autism Spectrum Disorder: Challenges and Future Directions. *J Med Chem.* **2017**;*60*:9114.

The serotonin system and ASD

Neuroscience 321 (2016) 24–41

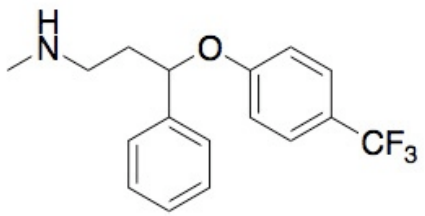
REVIEW

THE SEROTONIN SYSTEM IN AUTISM SPECTRUM DISORDER: FROM BIOMARKER TO ANIMAL MODELS

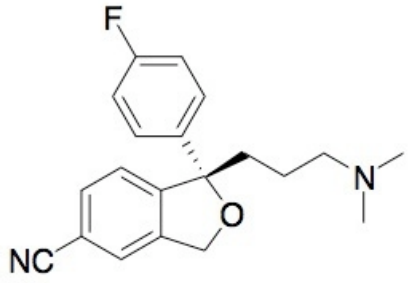
C. L. MULLER,[a] A. M. J. ANACKER[b] AND J. VEENSTRA-VANDERWEELE[c*]

- Platelet hyperserotonemia: ~70% increase of 5-HT level in platelet is observed in ~30% of ASD patients
- Various serotonin-related genes has been associated to ASD in humans
- Serotonin function is important in postnatal brain development

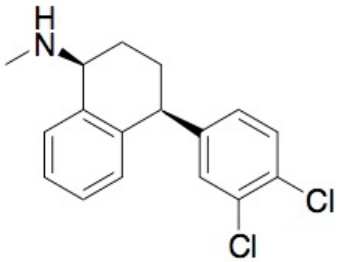
Selective Serotonin Re-uptake Inhibitors as "Off label" medicines



Fluoxetine

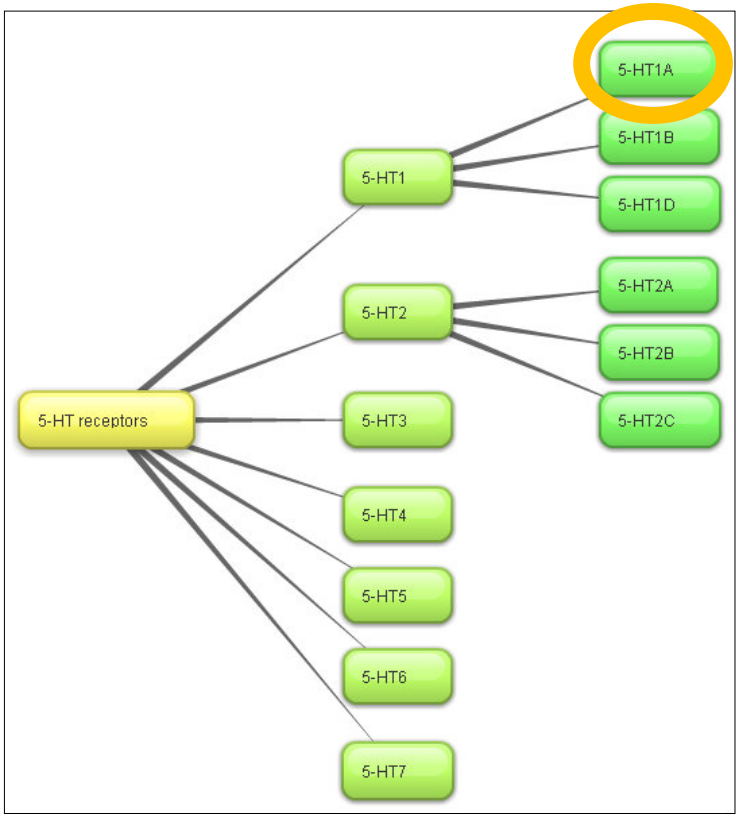


Escitalopram



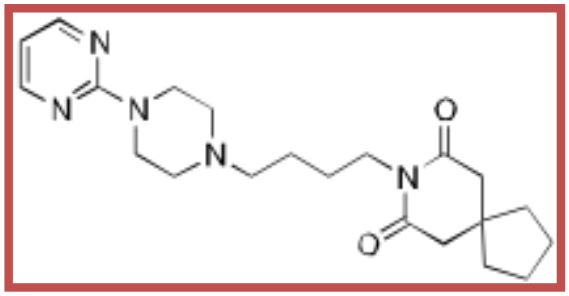
Sertraline

Targeting Serotonin Receptors in ASD



5-HT_{1A} receptor agonist treatment alleviate a reversal learning deficit in a mouse model of schizophrenia (McLean et al. 2009; Rajagopal et al. 2016)

Tandospirone reduces marble burying behavior in wistar rats (Abe et al. 1998)



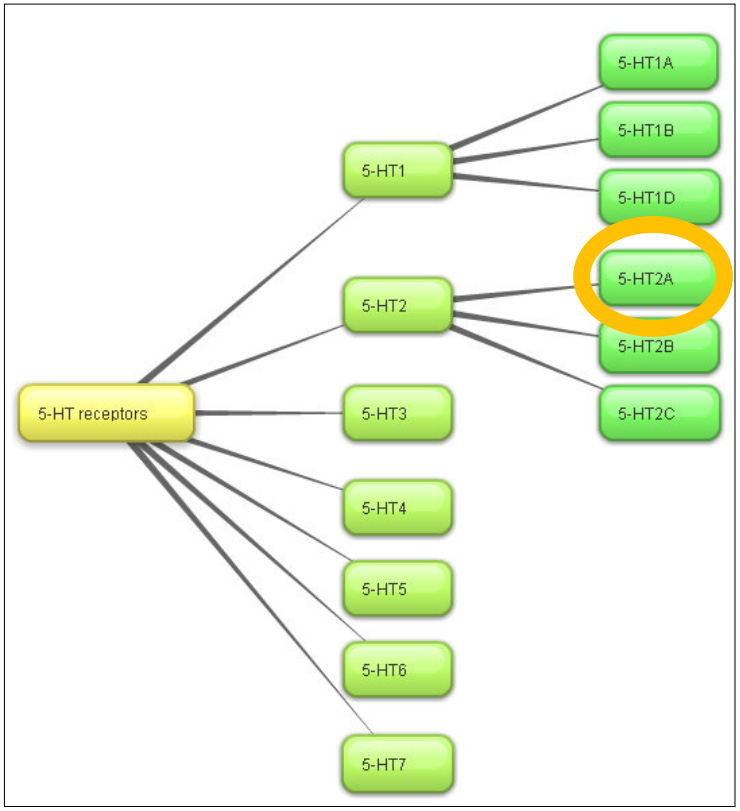
Buspirone

J Pediatr 2016;170:45-53

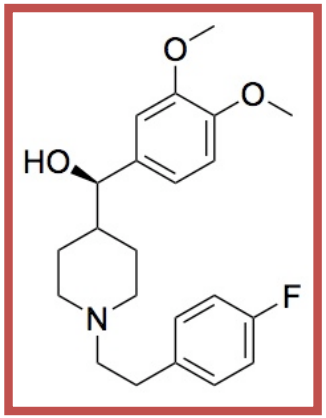
Efficacy of Low-Dose Buspirone for Restricted and Repetitive Behavior in Young Children with Autism Spectrum Disorder: A Randomized Trial

Diane C. Chugani, PhD[1,2], Harry T. Chugani, MD[1,2,3], Max Wiznitzer, MD[4], Sumit Parikh, MD[5], Patricia A. Evans, MD, PhD[6], Robin L. Hansen, MD[7], Ruth Nass, MD[8,9], James J. Janisse, PhD[10], Pamela Dixon-Thomas, PhD[1], Michael Behen, PhD[1,2], Robert Rothermel, PhD[11], Jacqueline S. Parker, BSc[1,2], Ajay Kumar, MD, PhD[1,2,3,12], Otto Muzik, PhD[1,2,3,12], David J. Edwards, PharmD[13], and Deborah Hirtz, MD[14], on behalf of the Autism Center of Excellence Network*

Targeting Serotonin Receptors in ASD



Systemic administration of the selective 5-HT_{2A} receptor antagonist M100907 in BTBR mice facilitates set-shifting and alleviates both a reversal learning deficit and elevated grooming behavior.



M100907

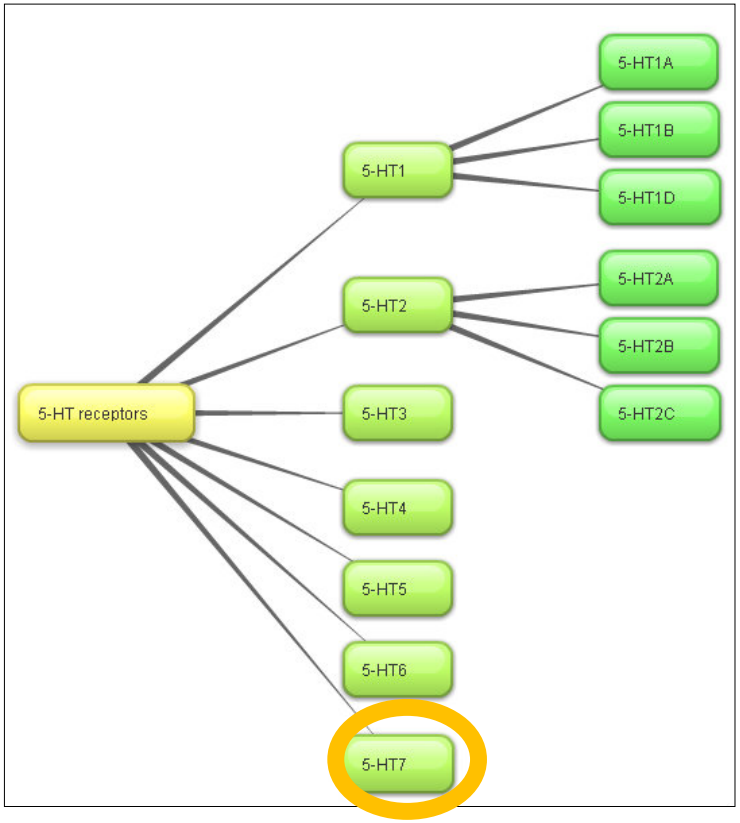
Genes, Brain and Behavior (2017) ***16:*** *342–351*

$5HT_{2A}$ receptor blockade in dorsomedial striatum reduces repetitive behaviors in BTBR mice

D. A. Amodeo[†,¶], E. Rivera[†], E. H. Cook Jr[‡], J. A. Sweeney[§] and M. E. Ragozzino[†,*]

Buspirone

Targeting Serotonin Receptors in ASD



BIOL PSYCHIATRY 2012;72:924–933

ARCHIVAL REPORTS

Activation of 5-HT7 Serotonin Receptors Reverses Metabotropic Glutamate Receptor-Mediated Synaptic Plasticity in Wild-Type and Fmr1 Knockout Mice, a Model of Fragile X Syndrome

Lara Costa, Michela Spatuzza, Simona D'Antoni, Carmela M. Bonaccorso, Chiara Trovato, Sebastiano A. Musumeci, Marcello Leopoldo, Enza Lacivita, Maria V. Catania, and Lucia Ciranna

Front. Behav. Neurosci. 9:65.

Novel agonists for serotonin 5-HT$_7$ receptors reverse metabotropic glutamate receptor-mediated long-term depression in the hippocampus of wild-type and Fmr1 KO mice, a model of Fragile X Syndrome

*Lara Costa[1], Lara M. Sardone[2], Enza Lacivita[3], Marcello Leopoldo[3] and Lucia Ciranna[2]**

Front. Behav. Neurosci. 9:86.

Long-lasting beneficial effects of central serotonin receptor 7 stimulation in female mice modeling Rett syndrome

Bianca De Filippis[1], Valentina Chiodi[2], Walter Adriani[1], Enza Lacivita[3], Cinzia Mallozzi[1], Marcello Leopoldo[3], Maria Rosaria Domenici[2], Andrea Fuso[4,5] and Giovanni Laviola[1]**

Neuropsychopharmacology (2014), 1–13

www.neuropsychopharmacology.org

Pharmacological Stimulation of the Brain Serotonin Receptor 7 as a Novel Therapeutic Approach for Rett Syndrome

Bianca De Filippis[1], Paola Nativio[2], Alessia Fabbri[3], Laura Ricceri[1], Walter Adriani[1], Enza Lacivita[4], Marcello Leopoldo[4], Francesca Passarelli[2], Andrea Fuso[5,6] and Giovanni Laviola[*,1]

[1]Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy; [2]Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy; [3]Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, Rome, Italy; [4]Department of Pharmacy, University of Bari 'A Moro', Bari, Italy; [5]Department of Psychology, Section of Neuroscience, Sapienza University of Rome, Rome, Italy; [6]European Center for Brain Research (CERC)/IRCCS Santa Lucia Foundation, Rome, Italy

Neuropharmacology 121 (2017) 79–88

Stimulation of the brain serotonin receptor 7 rescues mitochondrial dysfunction in female mice from two models of Rett syndrome

Daniela Valenti [a, **], Lidia de Bari [a], Daniele Vigli [b], Enza Lacivita [c], Marcello Leopoldo [c], Giovanni Laviola [b], Rosa Anna Vacca [a, 1], Bianca De Filippis [b, *, 1]

Buspirone

Targeting Multiple Serotonin Receptors in ASD: our hypothesis

Dual 5-HT_7/5-HT_{1A} (partial) agonists

- **increase social interaction** through activation of 5-HT_{1A} receptor
- **reduce stereotypy** and/or **improve cognition** through activation of 5-HT_7 receptor

Mixed 5-HT_{1A} /5-HT_7 agonist/5-HT_{2A} antagonist

- **improve social behavior** through activation of 5-HT_{1A} receptor
- **reduce or eliminate stereotyped behavior** by blocking 5-HT_{2A} receptor
- **improve cognition** through activation of 5-HT_7 receptor

How to design: Dual 5-HT_7/5-HT_{1A} (partial) agonists ?

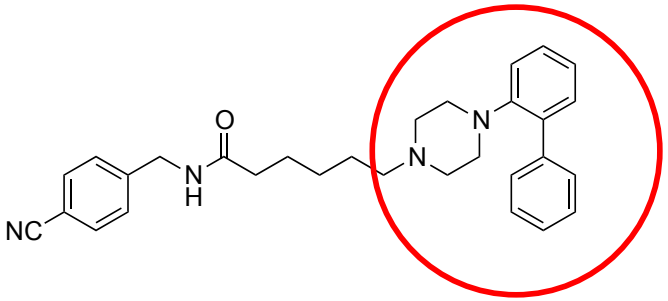
Mixed 5-HT_{1A} /5-HT_7 agonist/5-HT_{2A} antagonists ?

Terminus-Intermediate chain-PIPERAZINE-Ar

KNOWLEDGE-BASED DESIGN

Combination of fragments responsible for the desired activity at 5-HT receptors

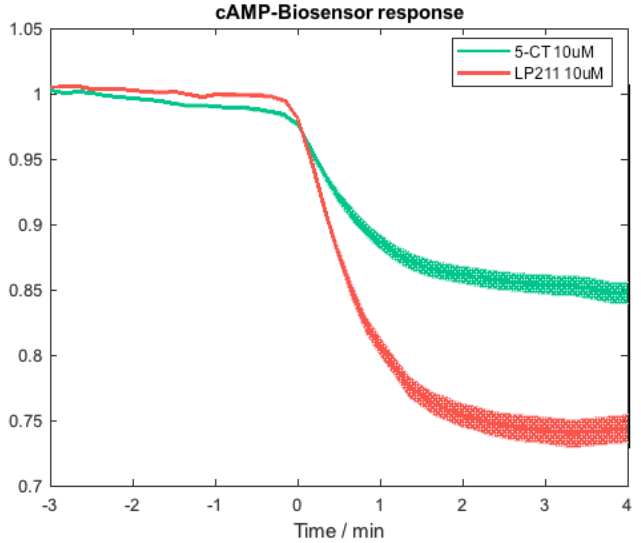
Structural motif for agonist activity at 5-HT$_7$ receptor



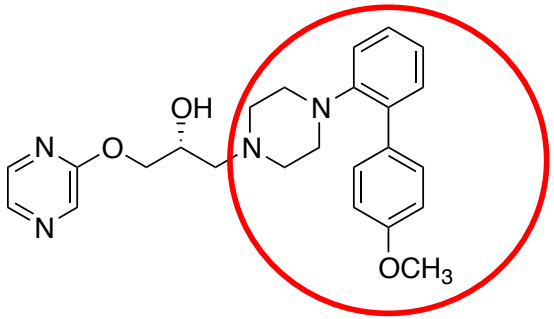
K_i [nM]		
5-HT$_7$	5-HT$_{1A}$	5-HT$_{2A}$
15	379	626

LP-211

Hedlund et al. Neurosci Lett. 2010

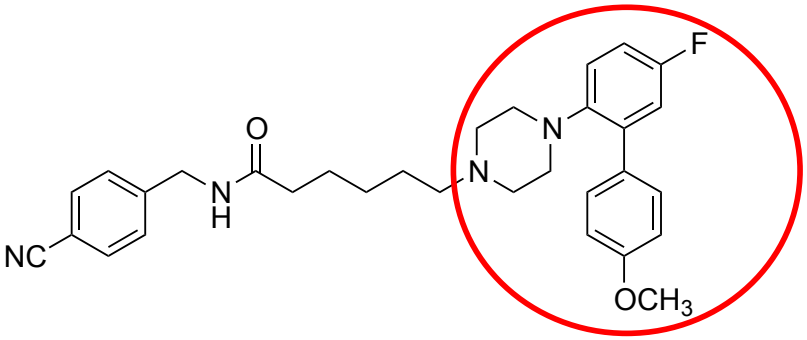


LP-211 stimulates cAMP production in 5-HT$_7$ receptor-expressing N1E-115 cells (Lacivita et al. 2020, submitted)



BA-10

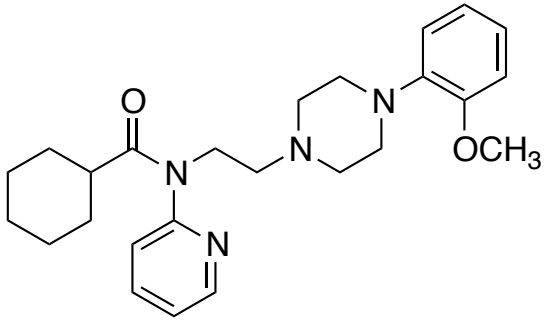
Costa et al. Front Behav Neurosci. 2015



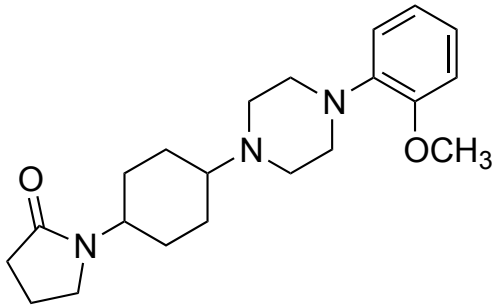
TP-22

Lacivita et al. Eur J Med Chem. 2016

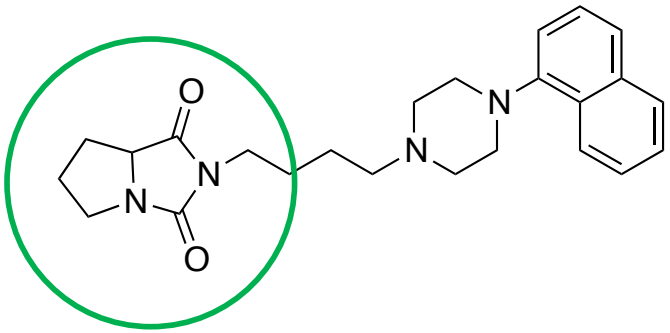
Structural motif for agonist activity at 5-HT_{1A} receptor



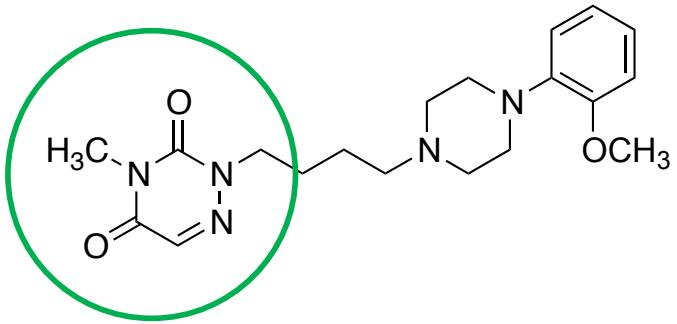
WAY-100635 (antagonist)
Forster et al. Eur J. Pharmacol. 1995



compound 16 (partial agonist)
Bojarski et al. Bioorg Med Chem. 2006

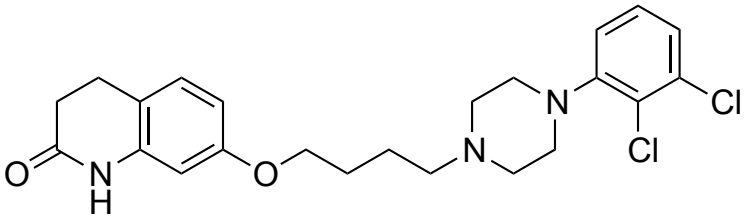


UCN-2550 (agonist)
López-Rodríguez et al. J Med Chem. 2005

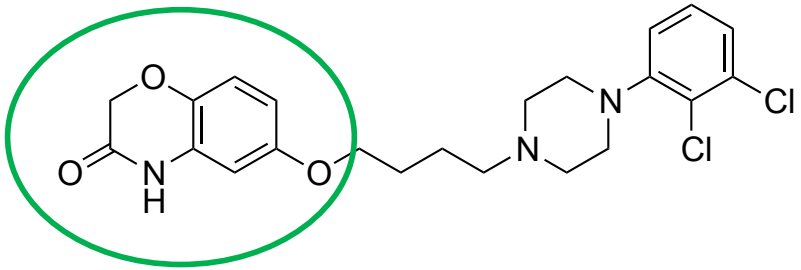


MMP (CUMI-101) (agonist)
Kumar et al. Eur. J. Nucl. Med. Mol. Imaging 2007

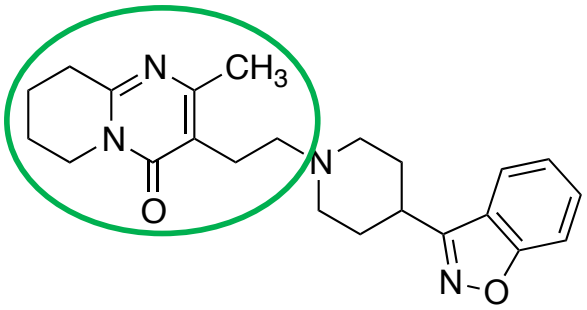
Structural motif for antagonist activity at 5-HT_{2A} receptor



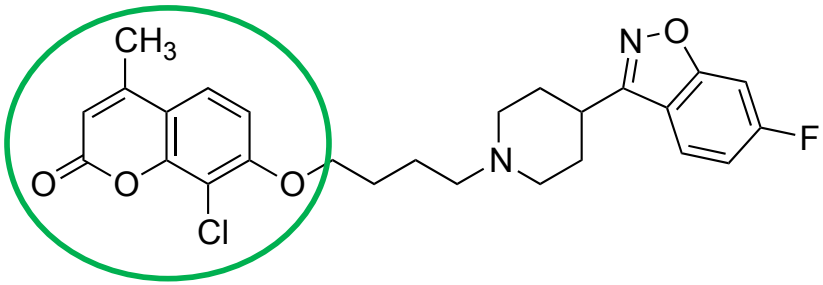
Aripiprazole (antagonist)
Forster et al. Eur J. Pharmacol. 1995



Brilaroxazine (antagonist)
Cantillon et al. Schizophr Res. 2017



Risperidone (antagonist)
Forster et al. Eur J. Pharmacol. 1995



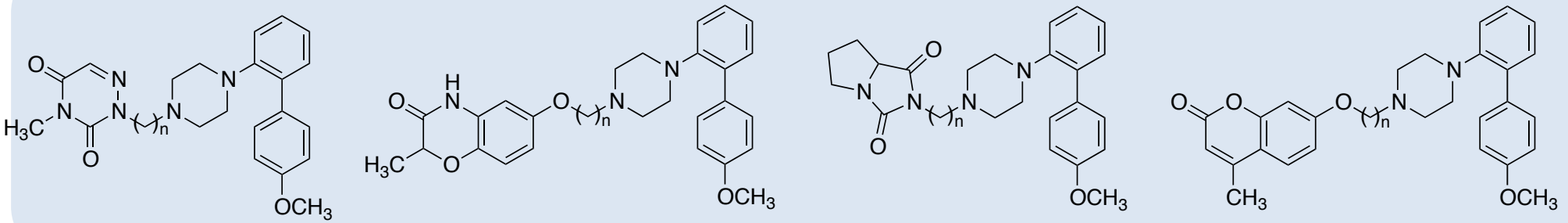
Compound 14m (antagonist)
Chen et al. J Med Chem. 2013

Terminus-Intermediate chain-PIPERAZINE-Ar

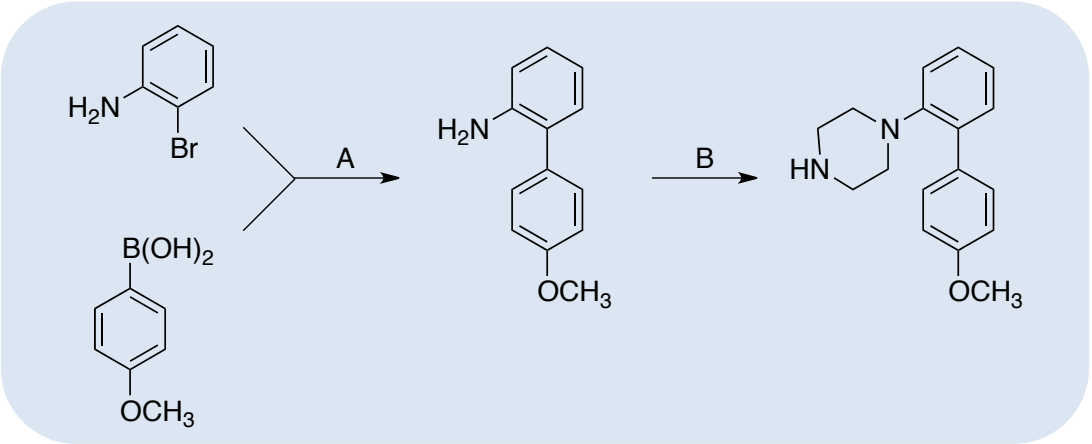
KNOWLEDGE-BASED DESIGN

Combination of fragments responsible for the desired activity at 5-HT receptors

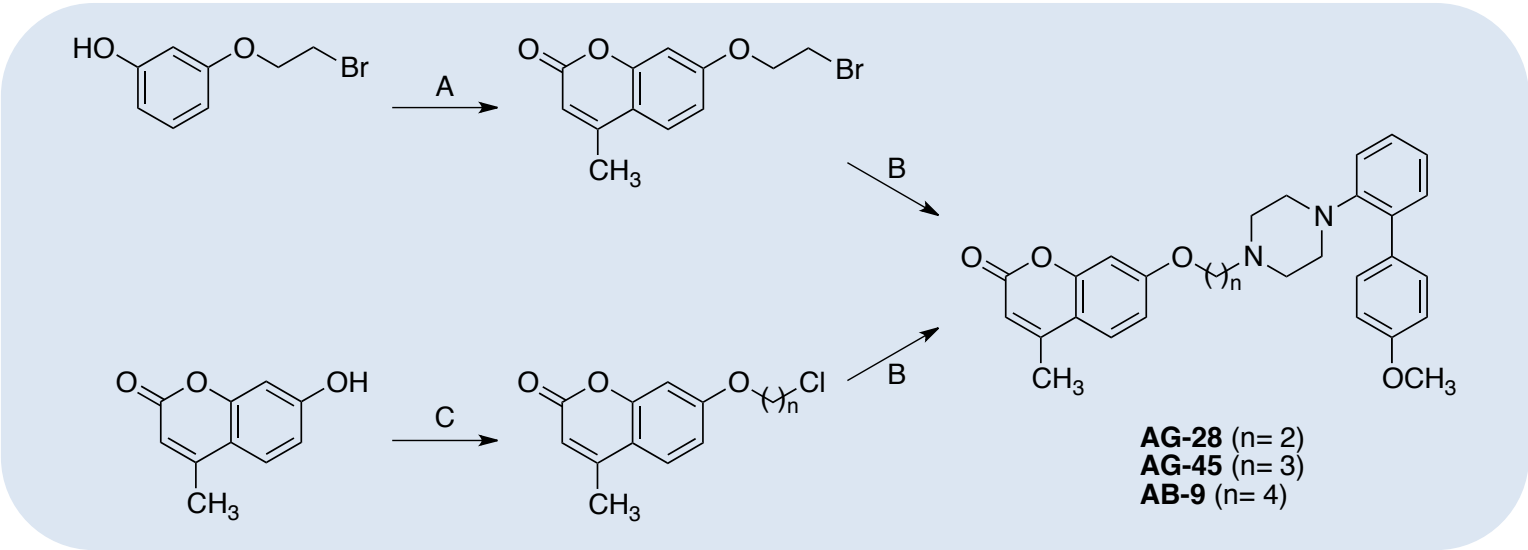
Target compounds



Synthesis of the Target Compounds



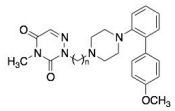
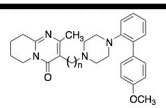
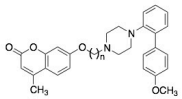
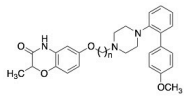
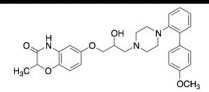
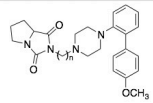
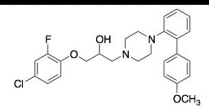
Reagents: A) $Pd(dppf)Cl_2$; 2M Na_2CO_3; B) bis(2-chloroethyl)amine·HCl, K_2CO_3, KI



Reagents: A) ethyl acetoacetate; conc. H_2SO_4; B) 1-arylpiperazine; K_2CO_3; C) NaH, $Br–(CH_2)_n–X$

Results and Discussion

Radioligand Binding and in vitro Metabolic Stability Data

Cmpd	Structure		MPO[a]	K_i [nM]				K_i ratio			MS[b] (%)
				5-HT$_{1A}$	5-HT$_7$	5-HT$_{2A}$	D$_2$	5-HT$_{1A}$/5-HT$_7$	5-HT$_{2A}$/5-HT$_7$	5-HT$_{2A}$/5-HT$_{1A}$	
AG4		n= 2	5.24	1721	80.0	2350	6577	22	29	1.4	17
AG44		n= 3	5.32	358	11.2	90.8	2084	32	8.1	0.25	< 2
ST58		n= 4	4.74	3.77	13	117	508	0.3	9	31	28
AG14		n= 2	3.91	289	25.6	73.5	592	11	2.9	0.25	21
ST143		n= 2	3.64	673	15.6	6.50	2972	43	0.41	0.01	14
AG28		n= 2	3.21	1761	91.7	220	301	19	2.4	8	< 2
AG45		n= 3	3.11	51.6	47.3	44.9	330	1.1	0.9	0.9	39
AB9		n= 4	2.76	135	42.9	54.7	147	3	1.3	0.2	20
AG16		n= 2	3.38	1802	57.2	312	541	32	5.5	0.2	28
AG47		n= 3	3.30	23.2	17.7	130	196	1.3	7.3	5.6	58
ST71		n= 4	2.94	127	14.7	107	82.8	9	7.3	0.65	49
ST72			3.44	8.70	19.9	141	419	0.44	7	16	68
AG27		n= 2	5.06	1648	51.6	2218	4955	32	43	1.3	< 2
AG26		n= 3	5.20	290	6.69	36.7	2148	43	5.5	8	18
MS12			3.04	264	44.3	310	845	6	7	1.2	34.6

[a] MPO: Multiparameter Optimization

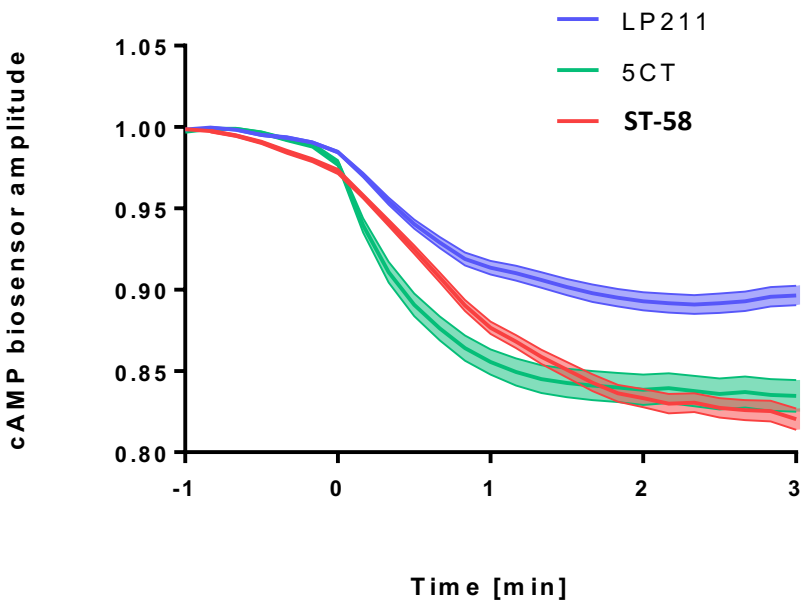
[b] MS: In vitro Microsomal Stability (% of recovery of the parent compound after 30 min incubation with rat microsomes)

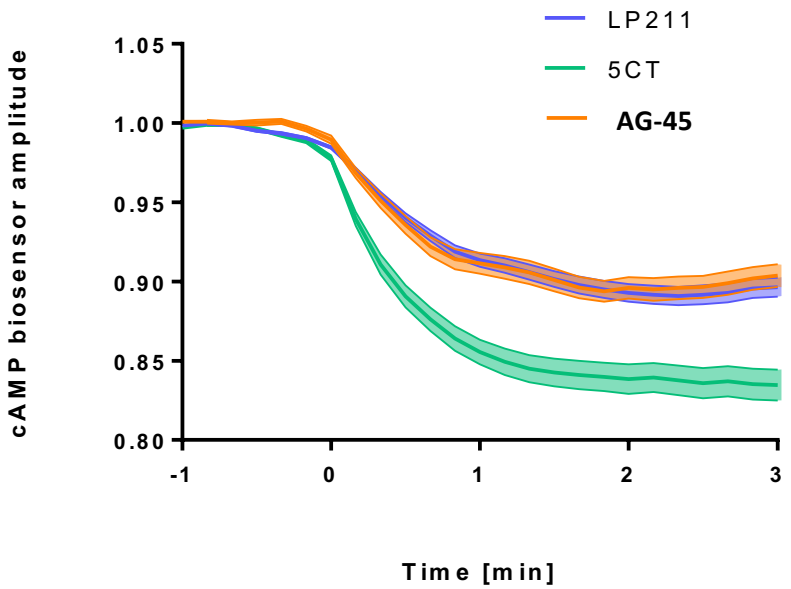
Half-life and Intrinsic Clearance of Selected Compounds

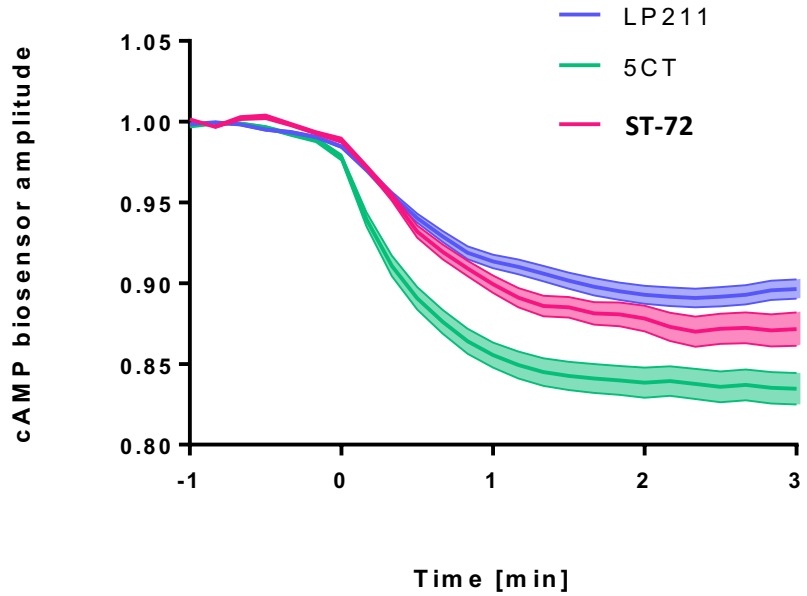
Compound	$t_{1/2}$ (min)	CL_{int} (µL/mg/min)
LP-211	15	45.9
TP-22	45	16.1
ST-58	41	16.9
AG-45	39	17.7
AB-9	23	30
AG-16	49	14.1
AG-47	60	11.5
ST-71	63	11
ST-72	74	9.4
MS-12	58	12

The data indicate that all the selected compounds showed higher stability than LP-211, with intrinsic clearance values lower up to 5-fold as in the case of compound ST-72. Thus, these compounds are predicted to be low-clearance compounds and suitable for studies in vivo

Functional study at 5-HT$_7$ receptor (cAMP signalling)

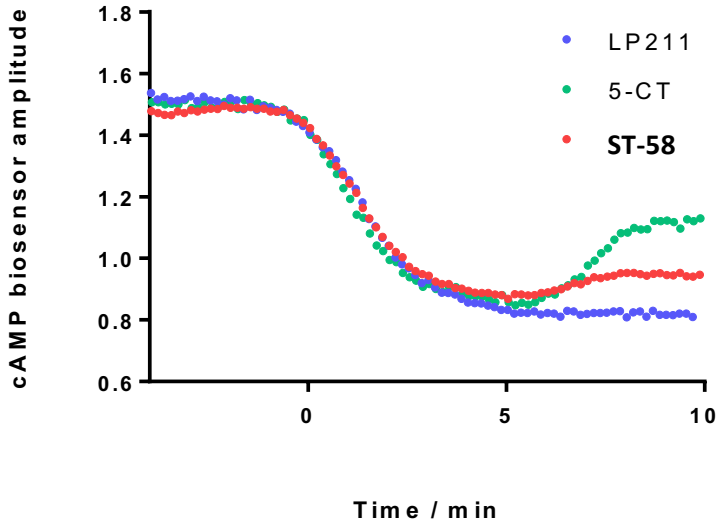


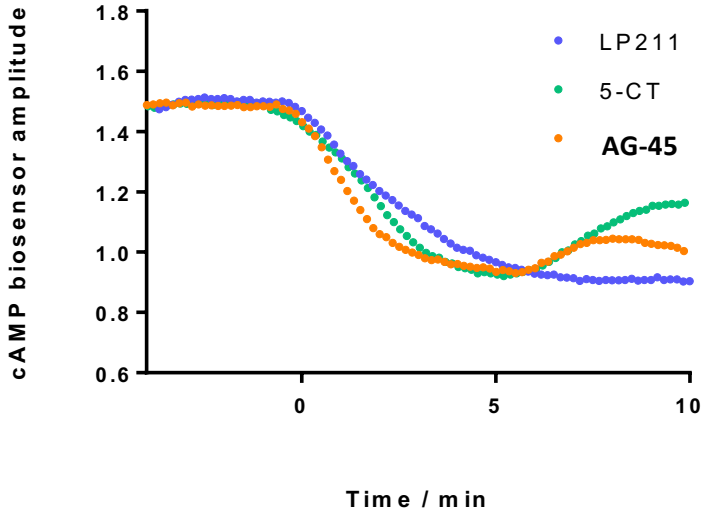


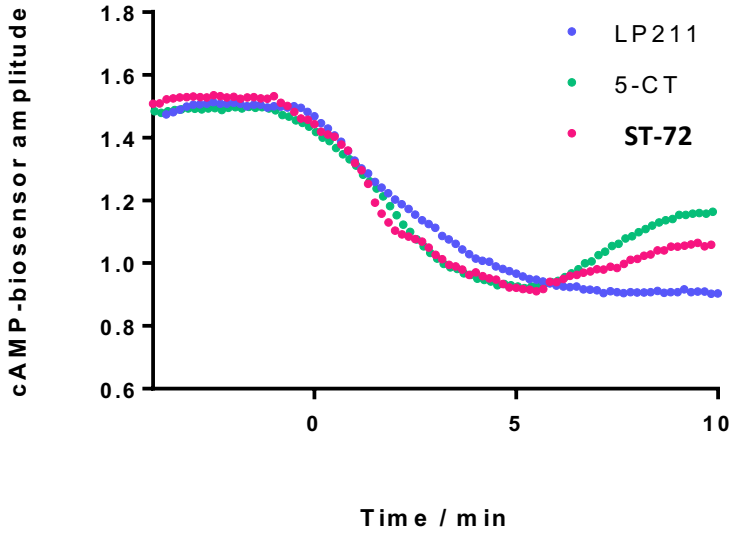


Compounds **ST-58**, **AG-45**, and **ST-72** stimulate 5-HT$_7$ receptor-mediated cAMP production. N1E cells were transfected with cAMP FRET-based biosensor CEPAC and 5-HT$_7$R-mCherry. Cells were stimulated with the compounds, as indicated. Mean values of the cAMP-biosensor response upon stimulation with **ST-58**, **AG-45**, and **ST-72** are shown. LP-211 and 5-CT were used as controls.

Functional study at 5-HT_{1A} receptor (cAMP signalling)

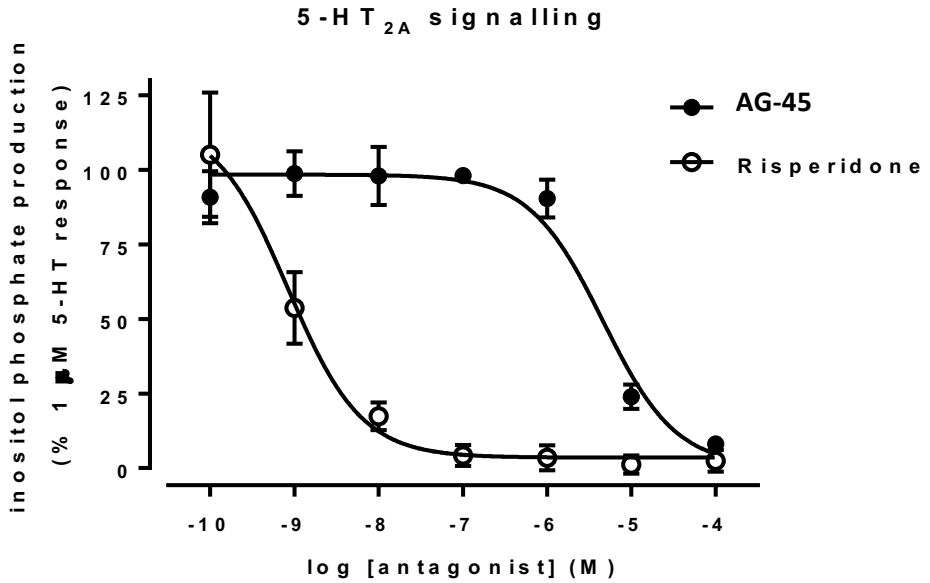






Compounds **ST-58**, **AG-45**, and **ST-72** behave as 5-HT_{1A} receptor agonists in the receptor-mediated cAMP inhibition. N1E cells were transfected with cAMP FRET-based biosensor CEPAC and 5-HT_{1A} receptor-mCherry. After pre-treatment with 1 µM forskolin and 25 µM IBMX, cells were stimulated with the indicated compounds. Each trace shows cAMP response at the single cell.

Functional study at 5-HT_{2A} receptor (inositol phosphate signalling)

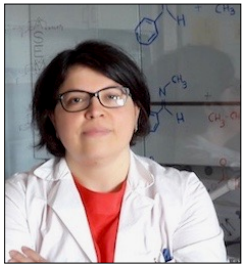


Concentration-response inhibition curves of **AG-45** and risperidone (as reference 5-HT_{2A} receptor antagonist) on inositol phosphate production stimulated by 1 µM 5-HT in CHO-K1 cells expressing human 5-HT_{2A} receptors.

Conclusions

- 5-HT neurotransmission system is an active area of investigation in ASD research since 1961
- SSRIs are efficacious to treat obsessive-compulsive disorder
- However, clinical studies indicate that SSRIs are not effective on the core symptoms of ASD
- Literature data suggest the investigation of new combinations of activities at 5-HT receptors
- We have identified new compounds with dual 5-HT_{1A}/5-HT_7 agonist properties (ST-58, ST-72) and the mixed 5-HT_{1A}/5-HT_7 agonist/5-HT_{2A} antagonist characteristics (AG-45)
- These compounds are metabolically stable in vitro and have suitable CNS drug-like properties
- Behavioral studies in animal models of ASD are in progress

Acknowledgments



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