Journal of Medicinal Chemistry

Perspective

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Targets for Drug Therapy for Autism Spectrum Disorder: Challenges and Future Directions

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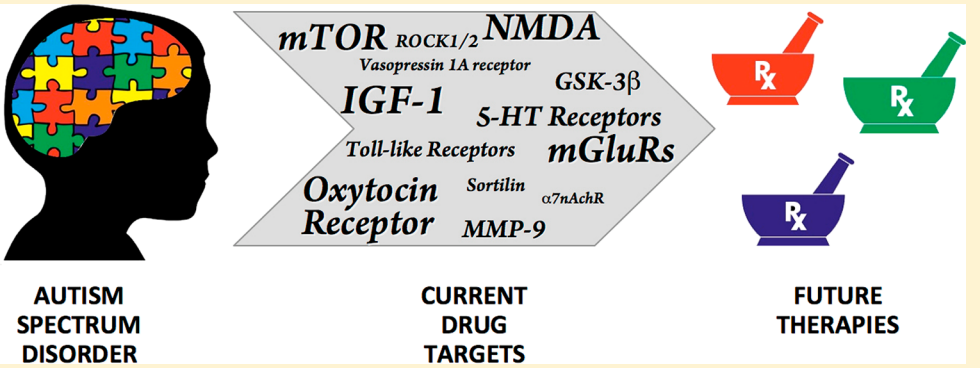

ABSTRACT: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by persistent deficits in social communication and interaction and restricted, repetitive patterns of behavior, interests, and activities. Various factors are involved in the etiopathogenesis of ASD, including genetic factors, environmental toxins and stressors, impaired immune responses, mitochondrial dysfunction, and neuroinflammation. The heterogeneity in the phenotype among ASD patients and the complex etiology of the condition have long impeded the advancement of the development of pharmacological therapies. In the recent years, the integration of findings from mouse models to human genetics resulted in considerable progress toward the understanding of ASD pathophysiology. Currently, strategies to treat core symptoms of ASD are directed to correct synaptic dysfunctions, abnormalities in central oxytocin, vasopressin, and serotonin neurotransmission, and neuroinflammation. Here, we present a survey of the studies that have suggested molecular targets for drug development for ASD and the state-of-the-art of medicinal chemistry efforts in related areas.

1. INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that is typically recognized in early childhood and has a lifelong course. According to the latest diagnostic criteria, it is characterized by two core symptoms: (1) persistent deficits in social communication and social interaction, (2) restricted, repetitive patterns of behavior, interests, and activities.[1] The diagnosis is based on clinical observation and further established by standardized testing of the patient with the Autism Diagnostic Observation Schedule-2,[2] and/or by parental interview with the Autism Diagnostic Interview-Revised.[3] Thus, far, no behavioral, neuroimaging, electrophysiological, or genetic tests can specifically diagnose ASD. Comorbid conditions such as intellectual disability, seizures, and sleep problems are frequent, whereas anxiety, depression, and obsessive−compulsive disorder (OCD) are less frequent.[4,5]

ASD distinguishes from most other behavioral disorders for the impressive clinical and pathogenetic heterogeneity, which has led to the designation with the term ASD of a set of neurodevelopmental disorders with early onset in life, sharing autism as a common feature, but caused by separate processes.[6] Originally, ASD was believed to be relatively rare, but the prevalence rates have dramatically increased in the past decade, from approximately 4/10000 to 1/68 children.[7] Various reasons have been put forward to account for this dramatic increase, including broadening of the spectrum to include even milder forms, improved clinical detection, and higher public awareness.[8] As a result, ASD has recently emerged as a major public health issue worldwide.

Altered neurodevelopment during the first and second trimesters of prenatal life is believed to be an underlying neuropathological cause of ASD.[9] Post-mortem studies have unveiled neuroanatomic and cytoarchitectonic aberrations in various brain regions, including cerebellum, hippocampus, inferior olivary complex, amygdala, entorhinal cortex, fusiform gyrus, and anterior and posterior cingulate cortex, with increased growth of the frontal lobes, thinner cortical minicolumns, and increased dendritic spine density.[10] These

Received: June 30, 2017
Published: October 17, 2017

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

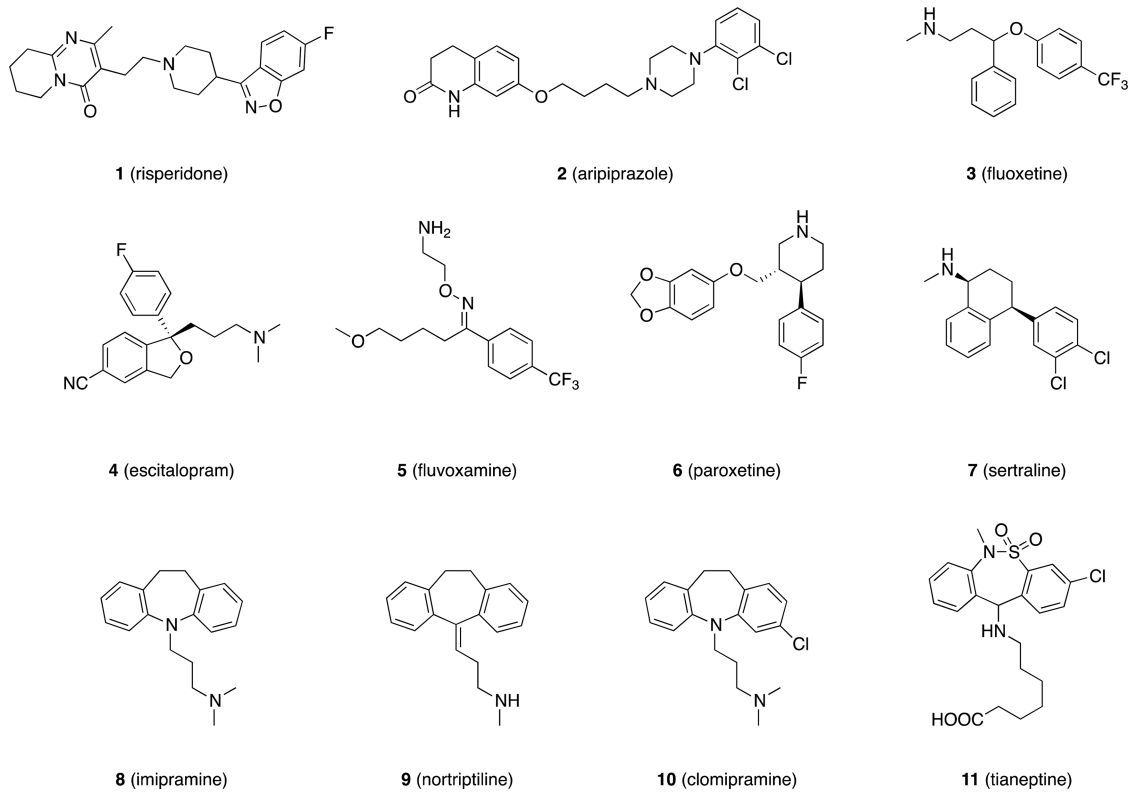

Figure 1. Antipsychotis and antidepressant drugs that are currently used in pharmacological treatment of ASD.

aberrations appear to be related to alterations occurring during early pregnancy, such as reduced programmed cell death and/or increased cell proliferation, altered cell migration, abnormal cell differentiation with reduced neuronal body size, abnormal neurite sprouting, and pruning that cause atypical wiring into the brain. In addition, because neurodevelopmental processes are still active into late prenatal and postnatal life, aberrations involve reduced synapse formation and delayed myelination.[11] The observed abnormal neuronal wiring was previously thought to be characterized by long-range hypoconnectivity and local hyperconnectivity.[12] Recent studies have instead shown that abnormal neuronal wiring is characterized by a highly individualized combination of hyper- and hypoconnectivity specific to each ASD patient.[13]

The neurocognitive phenotype of ASD is the result of a complex and highly heterogeneous set of genetic and environmental causes. However, in some patients, the disorder is the result of purely genetic causes due to known chromosomal aberrations or mutations,[14] while in other patients, the disorder is more likely related to environmental causes, such as prenatal exposure to chemical pollutants, toxins, viruses, or even drugs.[15] To date, hundreds of risk genes have been identified and not a major causative gene, with either rare variants that are highly penetrant or common variants with small effects.[14] It is therefore not surprising that this genetic heterogeneity is not associated with a characteristic neuropathology for ASD. Finally, neuroinflammation in ASD is receiving attention because of the altered expression of neuroinflammatory markers observed in the amniotic fluid, serum, cerebrospinal fluid, and the brain tissue of ASD patients.[16]

2. CURRENT PSYCHOTROPIC DRUGS ARE NOT EFFECTIVE TO TREAT CORE SYMPTOMS OF ASD

The use of pharmacotherapy as a component of treatment for ASD patients is common. However, psychotropic medications alleviate co-occurring psychiatric and behavioral problems, such as aggression, self-injury, impulsivity, hyperactivity, anxiety, and mood symptoms, but they do not have effect on the core symptoms of ASD.[17] The observed improvements in social interaction is a secondary effect of an overall reduction in maladaptive behaviors and not a primary effect of these medications.[17]

Benefits have been reported with (i) atypical antipsychotics for aggression, self-injurious behavior, or temper tantrums, (ii) selective serotonin reuptake inhibitors (SSRI) for anxiety and repetitive behaviors, and (iii) psychostimulants or opioid antagonists for hyperactivity.

To date, the only approved drugs to treat symptoms in ASD patients are compounds **1** (risperidone, Figure 1) and **2** (aripiprazole, Figure 1), both used to treat aggression, self-injury, and severe tantrums. Compound **1** is generally well tolerated, with no evidence of extrapyramidal side effects or seizures, whereas mild sedation, increased appetite, fatigue, dizziness, and drowsiness are frequent side effects. In addition, other side effects of this drug are metabolic alterations, including increased anthropometric and metabolic parameters, such as body mass index, waist circumference, and prolactin serum levels.[18−20] Compound **2** has similar effects but shows milder side effects which involve weight gain, fatigue and somnolence, gastrointestinal symptoms, and motor restlessness. Before the approval of compounds **1** and **2**, the second-generation antipsychotic agent clozapine was used for aggression and tantrums but has a limited usage because of the hematological safety concerns. Ziprasidone has also shown

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

some beneficial effects targeting irritability in ASD patients without any significant weight gain or other adverse effect.[21,22]

The SSRI **3** (fluoxetine, Figure 1) has shown various potential benefits, including reductions in rituals, stereotyped and repetitive behaviors in ASD patients. However, compound **3** produced effects like disinhibition, hypomania, agitation, and hyperactivity. Also, compounds **4** (escitalopram), **5** (fluvoxamine), **6** (paroxetine), and **7** (sertraline) (Figure 1) displayed the same potential benefits and adverse effects as **3**.[23] A recent review has highlighted the limited evidence of the effectiveness of SSRIs in adults.[24] Tricyclic antidepressants such as **8** (imipramine), **9** (nortryptiline), **10** (clomipramine), or **11** (tianeptine) (Figure 1) have been used in the treatment of ASD symptoms and comorbidities in ASD patients. However, limited and conflicting evidence emerged about either therapeutic effects or side effects of these medications.[25]

It has been proposed that anticonvulsants may be efficacious in the treatment of irritability and repetitive patterns of behavior in children with ASD (see ref 26 and references therein cited). However, compounds **12** (lamotrigine) and **13** (levetiracetam) (Figure 2) did not show efficacy in improving

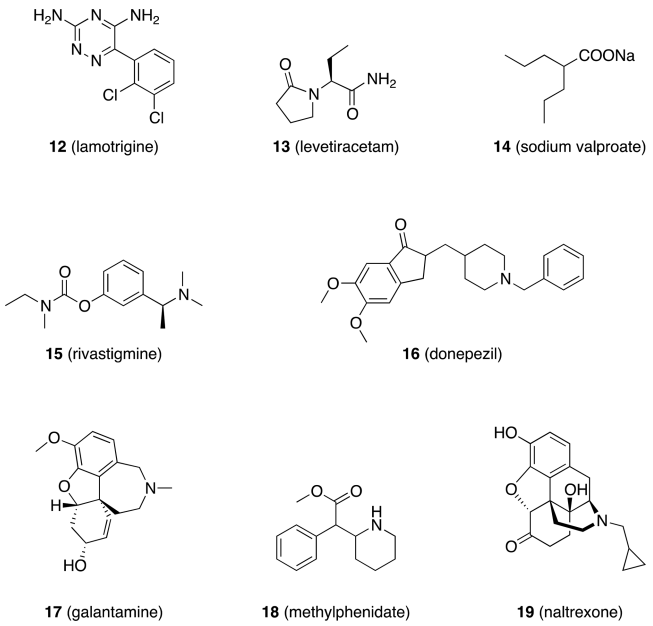

Figure 2. Psychotropic drugs that have been studied for ASD treatment.

ASD behaviors. On the other hand, compound **14** (valproate) (Figure 2) was found efficacious in the treatment of irritability and repetitive patterns of behavior in children with ASD.[26]

Because deficits in brain cholinergic function have been described in some ASD individuals,[27] the use of acetylcholinesterase inhibitors **15** (rivastigmine), **16** (donepezil), and **17** (galantamine) (Figure 2) has been tested in ASD children. Collectively, these studies reported some improvements in overall ASD behaviors and also in sleep patterns. Side effects include irritability, verbal or behavioral regression, headaches, rash, tremor, sedation, vomiting, and gastrointestinal problems.[28]

Compound **18** (methylphenidate, Figure 2) has been shown to be effective in improving attention deficit hyperactivity disorder (ADHD) symptoms in children with ASD. However, response rates are lower than those seen in typically developing children with ADHD. Moreover, discontinuation rates due to adverse events are high.[29]

The opiate antagonist **19** (naltrexone, Figure 2) has been evaluated in ASD patients on the basis of the hypothesis that endogenous opioids such as β-endorphin and enkephalins modulate social behavior.[30] Treatment with **19** improves self-injurious behavior, hyperactivity, social withdrawal, agitation, and irritability in ASD children.[31]

3. STRATEGIES TO TREAT CORE SYMPTOMS OF ASD

ASD has been classified into syndromic and nonsyndromic on the basis of clinical criteria. The term "syndromic" refers to conditions in which ASD occurs in conjunction with additional phenotypes and/or dysmorphic features. The etiology of syndromic ASD is known in most cases and can involve chromosomal abnormalities or mutations in a single gene. The study of syndromic ASD has yielded information on ASD at the molecular level on the pathways critical for cognitive and social development. Genetically modified mice based on human genetic findings have been crucial to deciphering previously unknown pathogenic mechanisms (for a recent review on ASD animal models see ref 32). These finding have led to the identification of potential targets for therapeutic intervention. Because new research reveals common features between syndromic and nonsyndromic forms of ASD, shared therapeutic approaches seem possible for this class of conditions.[33] We here recapitulate core phenotypes of syndromic forms of ASD that can be helpful for the reader.

Fragile X syndrome (FXS) is the most common genetic disorder associated with autism, affecting approximately 1/4000 males and 1/7000 females. FXS is caused by silencing of the *FMR1* gene, which encodes for the fragile X mental retardation protein (FMRP), an mRNA binding protein that functions as a regulator of protein synthesis and translation. FXS is characterized by intellectual disabilities, ranging from mild to severe, social anxiety and autistic disorders, such as stereotypical movements, increased susceptibility to seizures, attention deficit hyperactivity disorder symptoms, and sensory hypersensitivity.[34] The FXS animal model, the FMR1 knockout mice, recapitulates several behavioral and physical phenotypes of FXS observed in human patients.[35] These mice show increased density in dendritic spines, alteration in spine morphology,[36] and elevated metabotropic glutamate receptor-dependent long-term depression (mGluR-LTD). This latter observation has led to the mGluR theory of FXS.[37] In addition, FMR1 knockout mice show enhanced transmission activity of group I metabotropic glutamate receptor type 5 ($mGlu_5$)[35] and GABAergic deficits in several brain areas (cortex, hippocampus, amygdala, striatum, and subiculum).[38]

Rett Syndrome (RTT) is a rare neurodevelopmental disorder that affects 1/15000 women. RTT is due to loss-of-function mutations in the X-linked *MECP2*, a gene encoding a multifunctional protein that binds to methylated DNA and acts as a key transcriptional regulator. RTT is also associated with regression of language, cognitive functions, social and motor skills, stereotypies, seizures, and breathing difficulties. At the cellular level, the brains of RTT patients are characterized by reduced neuronal size, increased cell density in several regions, reduced dendritic arborization, low spine density, and altered spine morphology.[39] In addition, transgenic mice studies have reported that MeCP2 deficiency is critical for normal activity of GABA-releasing neurons and that

DOI: 10.1021/acs.jmedchem.7b00965
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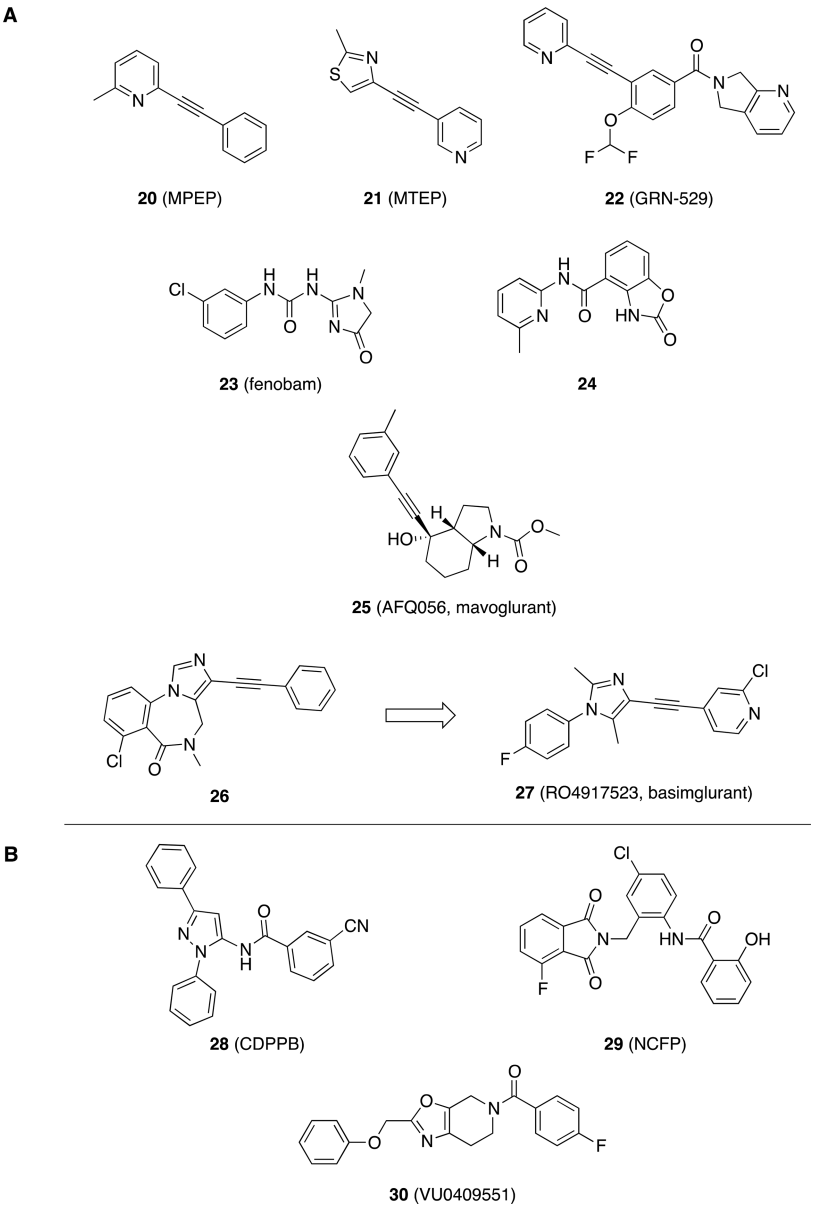

Figure 3. (A) Structure of $mGlu_5$ NAMs that have been studied in ASD models. (B) Structure of $mGlu_5$ PAMs.

dysfunctions of GABAergic neurons contributes to the altered behavioral phenotypes observed in RTT.[40]

Tuberous sclerosis complex (TSC) is a genetic disorder related to ASD characterized by the formation of hamartomas (tumor-like nodules) in multiple organ systems, including central nervous system (CNS), and affects 1/6000 individuals. TSC is associated with learning abnormalities, intellectual disabilities, developmental delay, autistic features, and epilepsy.[41] TSC is caused by mutations in either *TSC1* or *TSC2* genes encoding for hamartin and tuberin, respectively, two proteins that regulate the activity of the mammalian target of rapamycin (mTOR) pathway. In TSC, mTOR is hyperactive, and this translates into abnormal protein synthesis and synaptic plasticity, reduced neuronal connectivity and CNS myelination, and imbalance of synaptic excitatory/inhibitory (E/I) ratio. Moreover, loss of functional *TSC1*/*TSC2* genes affects dendritic spine formation and structure and dendritic arborization.[42]

3.1. Targeting Synaptic Dysfunction. Several lines of evidence indicate that disrupted synaptic function appears to be a basis of ASD pathophysiology. Several genes associated with ASD encode proteins that directly or indirectly affect synaptic function. Impaired synaptic plasticity might lead to neuronal networks with reduced capacity to change their structure and function. Therefore, improving neurological deficits by enhancing synaptic plasticity in a way that is independent from the disorder-specific etiology can represent a valuable therapeutic strategy.[33]

3.1.1. Excitatory/Inhibitory (E/I) Balance. A widely accepted hypothesis on the etiology of ASD proposes that there is E/I imbalance in brain neural circuits.[43] E/I imbalance may be due to an increase of glutamatergic or to a decrease of GABAergic signaling and it may give rise to altered synaptic plasticity and learning and memory, seizures, neural network oscillatory abnormalities, visual system abnormalities, general dyspraxia, behavioral changes, and social dysfunction.[44] Yizhar et al. have demonstrated in mice that increased E/I ratio in the prefrontal

DOI: 10.1021/acs.jmedchem.7b00965
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cortex is related to behavioral and social impairments relevant to ASD.[45] On the other hand, decreased E/I ratio was observed in a mouse model of RTT.[46]

Different pharmacological approaches have been proposed to restore E/I imbalance.

Modulation of $mGlu_5$ Receptor. The "mGluR theory" of FXS implicated that blockade of $mGlu_5$ receptor could be useful to treat the neurological and psychiatric symptoms of FXS,[37] and preclinical studies have supported this theory (for a comprehensive review see ref 47 and references therein cited).

The design of competitive ligands for mGlu receptors has been challenging due to the difficulty to identify selective molecules. Competitive ligands for mGlu receptors are usually polar amino acid-like molecules with reduced ability to cross the blood−brain barrier (BBB). Instead, allosteric modulators of $mGlu_5$ receptor (positive allosteric modulators, PAMs, or negative allosteric modulators, NAMs) are more drug-like small molecules structurally unrelated to glutamate and allow fine-tuning of the signal transduction toward predefined level.[48]

The potent, selective, and brain penetrant mGlu5 NAMs **20** (MPEP) and **21** (MTEP) (Figure 3) have been two milestones in this research field.[48] In fact, treatment of FMR1 knockout mice with **20** suppressed the audiogenic seizure phenotype, rescued prepulse inhibition, and reduced repetitive autistic-like behavior. In addition, administration of compound **20** rescued the immature morphological phenotype of pyramidal neurons in the somatosensory cortex of neonatal FMR1 knockout mice.[47]

In BTBR mice, a well-validated model of idiopathic autism,[32] compound **20** significantly reduced repetitive self-grooming without inducing sedation on open field activity but did not improve sociability.[49] In the BTBR and C58 mouse strains, the $mGlu_5$ NAM **22** (GRN-529, Figure 3) suppressed repetitive behaviors and social behavior deficits.[50]

In addition, compound **20** normalizes learning measures in BTBR mice (hippocampus-dependent object location memory). In contrast, semichronic treatment with the AMPA receptor PAM ampakine, which facilitates memory in other models of cognitive impairment, had no effect on object location memory in BTBR mice.[51] Moreover, compound **20** significantly reduced elevated stereotyped, repetitive, and anxiety-like behaviors in the valproic acid mouse model of ASD.[52]

Besides compounds **20** and **21**, a number of $mGlu_5$ NAMs have been reported in the literature, showing structures different from the diarylethynyl scaffold (for extensive reviews see refs 53 and 54). Here, the $mGlu_5$ NAMs studied in ASD context are illustrated. Compound **23** (fenobam, Figure 3) was able to rescue abnormalities in neuronal morphology in FMR1 knockout mice.[55] Using the structure of **23** as a template, Hoffmann-La Roche developed a series of benzoxazolones, exemplified by compound **24** (Figure 3), as $mGlu_5$ NAMs with good pharmacokinetic (PK) properties and activity in anxiety models after oral administration.[56]

Compound **25** (AFQ056 or mavoglurant, Figure 3) was identified by Novartis via an HTS campaign focused on the identification of $mGlu_5$ NAMs structurally different from **20**. Compound **25** was characterized as a potent and selective $mGlu_5$ NAM in vitro and demonstrated improved in vivo bioavailability in rats and reduced in vitro clearance in human microsomes as compared to **20**.[57] Compound **25** was able to rescue dendritic spine phenotype in FMR1 knockout mice.[47] However, randomized, double-blind, placebo-controlled studies with this compound in FXS adults did not allow confirmation of the results observed in animal models.[58]

Starting from the screening hit **26** (Figure 3), having weak $mGlu_5$ activity but potent $GABA_A$ agonistic activity, Roche identified the compound **27** (RO4917523 or basimglurant, Figure 3), which showed potent in vivo activity in preclinical models of anxiety, favorable PK properties in rats and monkeys, and an excellent preclinical safety profile.[59] Also, compound **27** entered clinical trials for the treatment of FXS, but the results did not confirm the results observed in animal models.[58]

The results of $mGlu_5$ receptor inhibitors in preclinical studies and proof-of-concept clinical trials generated high excitement. However, the trials failed to demonstrate sufficient significance. As highlighted in a detailed review on the clinical trials with investigational drugs for the treatment of FXS,[60] the differences in outcome between the animal models and humans have evidenced the unique challenges of carrying out trials in these cognitively and behaviorally challenged individuals as well as a paucity of clinically relevant outcome measures for use in these trials.

Results with compound **28** (CDPPB, Figure 3), an $mGlu_5$ PAM, in animal models of ASD (see below) have revived the interest in the development of PAMs. For many years, the development of $mGlu_5$ PAMs has not been pursued because they can induce potent activation of $mGlu_5$ signaling, which can in turn induce epileptiform activity, seizures, and neurotoxicity.[61] However, compound **29** (NCFP, Figure 3) was found to positively modulate $mGlu_5$ without the induction of LTD and LTP in the hippocampus, suggesting that the compound stabilizes a unique active receptor conformation.[61] Moreover, the adverse effect liability of $mGlu_5$ PAMs is thought to be related to the NMDA receptor. Therefore, the identification of PAMs displaying signal bias away from potentiation of NMDA activation offers an alternative for modulating $mGlu_5$ receptor signaling without inducing neurotoxicity.[62] Researchers at Vanderbilt University reported the compound **30** (VU0409551, Figure 3) as a potent and orally bioavailable $mGlu_5$ PAM that displays robust antipsychotic and cognition-enhancing efficacy in the absence of direct potentiation of NMDA receptor.[63]

Modulation of Group II Metabotropic Glutamate Receptors. Group II metabotropic glutamate receptors ($mGlu_2$ and $mGlu_3$) have also been proposed as potential targets for therapeutic intervention in ASD. Chen et al. have reported that activation of $mGlu_{2/3}$ may underlie the effects of *N*-acetylcysteine on amygdala-associated autism-like phenotypes in a valproate-induced rat model of autism.[64]

Several classes of compounds have been described as modulators of $mGlu_2$ and $mGlu_3$ which have proved to be effective in various preclinical models of CNS disorders, such as schizophrenia and OCD, but none have been studied in preclinical models of ASD (for an extensive review see ref 48 and references herein cited).

Orthosteric agonists and antagonists have been developed starting from glutamate as lead structure. Eli Lilly described the bicyclo[3.1.0]hexane **31** (LY354740 or eglumetad) and its closely related ether analogue **32** (LY379268) as prototypical $mGlu_{2/3}$ orthosteric agonist tools (Figure 4).[65] Two highly functionalized glutamate analogues, compounds **33** (LY341495) and **34** (MGS0039) (Figure 4), have been used to evaluate the potential of $mGlu_{2/3}$ blockade to treat OCD, anxiety, and cognitive deficits.[66,67] In addition, several $mGlu_{2/3}$ PAMs have been developed as an alternative to orthosteric

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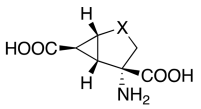
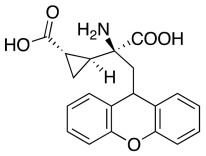
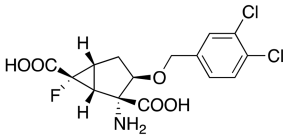


31 X = CH_2 (LY354740, eglumetad)
32 X = O (LY379268)



33 (LY341495)



34 (MGS0039)

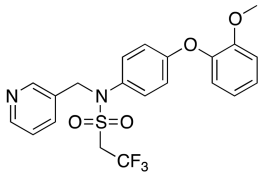
Figure 4. Structures of orthosteric agonists and antagonists of $mGlu_{2/3}$.

agonists. Prototypical $mGlu_2$ PAMs are two structurally unrelated compounds, i.e., the tertiary sulfonamide **35** (LY487379)[68] and the 2-cyclopentyl indanone **36** (BINA)[69] (Figure 5). Researchers at Taisho Pharmaceuticals reported the characterization of the selective $mGlu_2$ PAM **37** (TASP0433864, Figure 4), which was useful in restoring E/I imbalance underlying schizophrenia.[70] Addex Pharmaceutical has developed the compound **38** (ADX71149 or JNJ-40411813, Figure 5), which demonstrated positive effects as adjunctive treatment to antipsychotics in patients with negative symptoms of schizophrenia.[71] Astra Zeneca has reported the isoindolinone **39** (AZD8529, Figure 5), which displayed antipsychotic properties in different preclinical models of schizophrenia.[72]

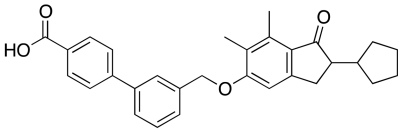
As for $mGlu_{2/3}$ NAMs, Roche reported a series of benzodiazepine derivatives exemplified by **40** (RO4491533, Figure 5), which proved to be effective in rodent models of depression and cognition.[73]

Modulation of Ionotropic Glutamate NMDA Receptors. $mGlu_5$ receptor and the ionotropic glutamate receptor NMDA show a positive reciprocal regulation, where activation of one of them potentiates the response elicited by the other one. Conversely, blocking one of them indirectly inhibits the other.[74] Thus, by extension of the findings described above for $mGlu_5$ antagonists, the NMDA receptor antagonist **41** (memantine, Figure 6) (approved by the U.S. Food and Drug Administration (FDA) for use in Alzheimer's disease) has been evaluated as pharmacotherapy in ASD. It was shown that either NMDA antagonism through compound **41** or $mGlu_5$ antagonism through **20** was able to rescue social deficits as well as NMDA hyperactivity in IRSp53 knockout mice, a gene linked to ASD in humans.[75] Conversely, in Shank2 knockout mice, another gene linked to ASD, which show decreased NMDA receptor function, the treatment with either the NMDA agonist **42** (D-cycloserine, Figure 6) or an $mGlu_5$ PAM is able to restore NMDA activity and social behavior.[76] Accordingly, clinical trials have shown that compound **42** improves social and repetitive behaviors in ASD patients.[77,78] Furthermore, clinical trials in individuals with ASD reported improvements with NMDA receptor antagonist **41**.[28]

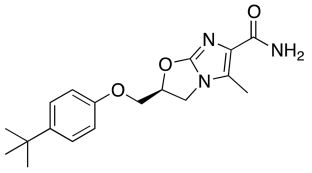
Recently, Volkman et al. have described the identification of the antagonists **43** (MPX-004) and **44** (MPX-007) (Figure 6) as pharmacological tools to study the function of the NMDA receptors containing a GluN2A subunit. Both compounds were able to antagonize GluN2A-containing NMDA receptors expressed in HEK cells. Electrophysiology studies demonstrated that maximal concentrations of both compounds



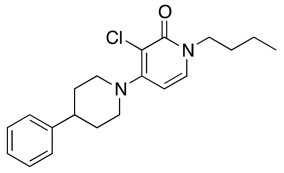
35 (LY487379)



36 (BINA)



37 (TASP0433864)



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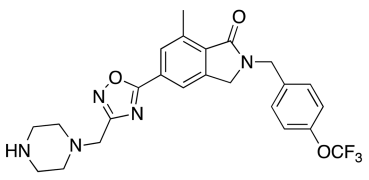

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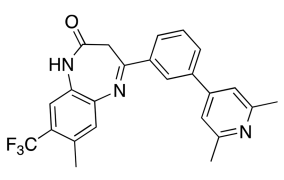

40 (RO4491533)

Figure 5. Structures of $mGlu_{2/3}$ PAMs and NAMs.

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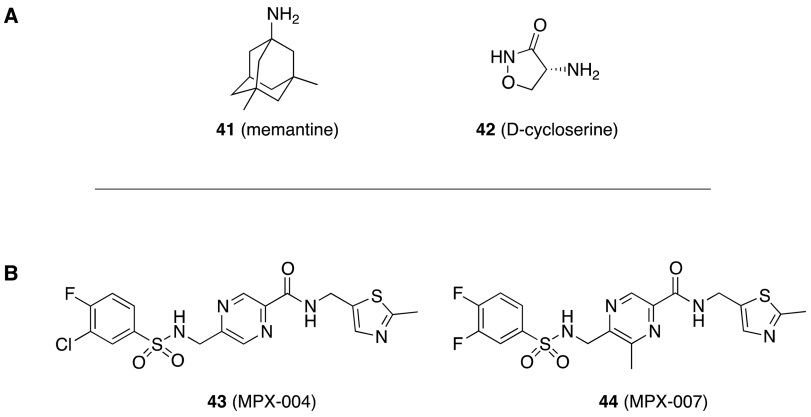

Figure 6. (A) NMDA antagonists that have studied in ASD animal models. (B) New NMDA antagonists.

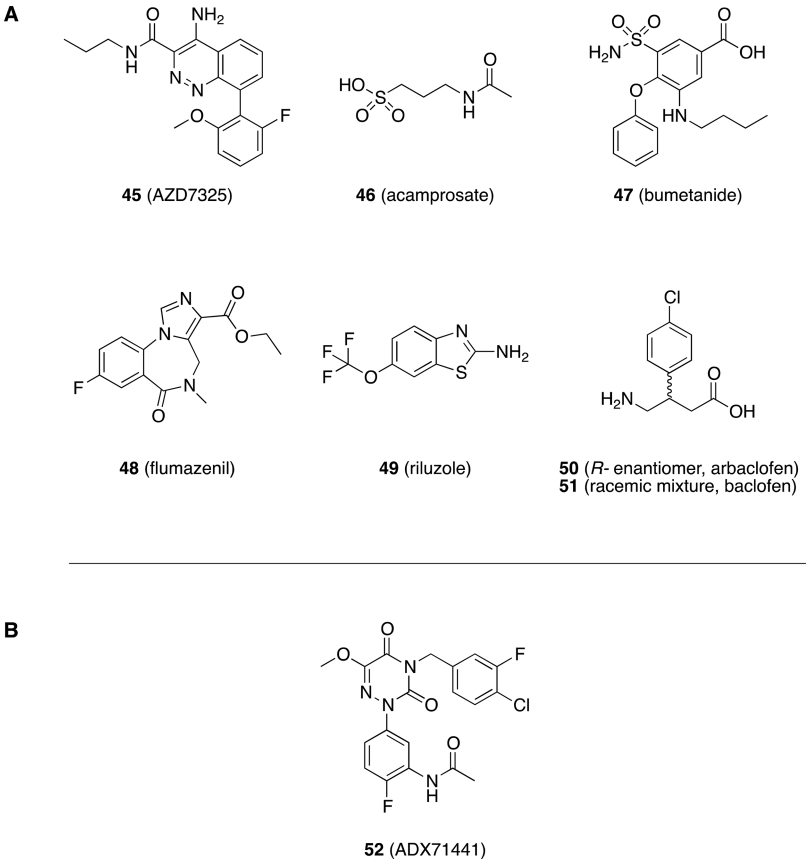

Figure 7. (A) Drugs modulating GABA transmission that have been subjected to clinical trials for ASD treatment. (B) $GABA_B$ PAMs.

inhibited the whole-cell current (~30%) in primary culture of rat pyramidal neurons and NMDA receptor-mediated EPSP (~60%) in rat hippocampal slices, suggesting that both **43** and **44** could be useful to study GluN2A involvement in neuropsychiatric and neurodevelopmental disorders.[79]

Modulation of GABAergic Activity. GABA is generally considered as the main inhibitory neurotransmitter that regulates the release of other neurotransmitters and neurons excitation. In 2001, Hussman proposed that several functional deficits in ASD might be linked to the suppression of the GABAergic inhibitory tone in several brain regions and that the loss of inhibitory control from GABAergic neurons might result in hyperexcitation of target neurons or selective vulnerability to glutamate.[80] Physiological effects of GABA are mediated through the interaction with three different receptor subtypes: $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ and $GABA_C$ are ligand-gated ion channels that suppress neuronal excitability. $GABA_B$ is an unusual G-protein-coupled receptor which is a heterodimer of $GABA_{B1}$ and $GABA_{B2}$ subunits. These receptors can inhibit the release of many neurotransmitters, such as dopamine, serotonin, and acetylcholine, via a G-protein-dependent inhibition of neuronal voltage-gated Ca^{2+} channels.[81]

Experimental evidence from in vitro and in vivo studies in animal models or in humans seem to support the hypothesis that an imbalance of either $GABA_A$, and $GABA_B$ receptor signaling may result in increased postsynaptic neuronal excitability and altered glutamate release.[82,83] Thus, increasing

DOI: 10.1021/acs.jmedchem.7b00965
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GABAergic transmission might improve behavior by compensating a potentially excessive glutamatergic neurotransmission. Both ionotropic and metabotropic GABA receptor subtypes represent potential targets for development of therapeutic agents. Interestingly, pharmacological enhancement of GABA neurotransmission is able to improve the core social interaction deficits in BTBR mice.[84] On the basis of these data, Astra Zeneca and NIH have initiated clinical trials of the $\alpha_{2,3}$-selective PAM of $GABA_A$ receptor **45** (AZD7325, Figure 7)[85] for treatment of social disability in young adults with ASD (NCT01966679). On the basis of these observations, various clinically approved drugs that modulate GABA transmission, such as **46** (acamprosate), **47** (bumetanide), **48** (flumazenil), **49** (riluzole), and **50** (arbaclofen) (Figure 7), have been subjected to clinical trials (for a recent review see ref 86). The clinical trials yielded mixed and inconclusive results. With this respect, the failure of compound **50** in clinical trials has been the major disappointment in ASD research.[87,88] The largest clinical trial on compound **50** failed to provide significant difference in the primary outcome measure (ABC-Social Withdrawal score) but provided significant improvement in secondary outcome measures (social impairment among others).[87] Possible explanations for the lack of efficacy of compound **50** (and other medications) could arise from the extreme heterogeneity of ASD. In fact, as the symptoms may differ significantly in quality and severity among patients, it could be difficult to measure significant changes with a single outcome.

To date, compound **51** (baclofen), Figure 7, is the only drug approved by the FDA which targets the $GABA_B$ receptor. However, compound **51** showed severe side effects, which include hypothermia, seizures, sedation, cognitive deficits, and drug tolerance. Compound **51** also exhibits poor brain penetrance and therefore requires high doses for engagement of the $GABA_B$ receptor in the CNS, resulting in elevated plasma concentrations and activation of peripheral $GABA_B$ receptors on smooth and skeletal muscle. Numerous $GABA_B$ agonists have been developed from compound **51** with the aim to improve potency and CNS penetration. Moreover, the scaffold of compound **51** has been manipulated to develop $GABA_B$ antagonists, which, however, show poor selectivity. Also for $GABA_B$ receptors, PAMs and NAMs have been developed as an alternative to orthosteric agonists and antagonists and tested in different behavioral paradigms. For an extensive review on orthosteric agonists and PAMs of $GABA_B$ receptor, see ref 89. Recently, the potent, selective, and brain penetrant $GABA_B$ PAM **52** (ADX71441, Figure 7) has been reported, showing efficacy in animal models of anxiety and pain. No data on ASD-relevant behavioral tests were reported.[90]

Glycogen Synthase Kinase 3 (GSK-3) Inhibition. GSK-3 is an evolutionarily conserved serine/threonine kinase highly abundant in the brain and acts as the main suppressor of the Wingless (Wnt)/β-catenin signaling pathway. The Wnt/β-catenin pathway plays a crucial role in the proliferation, differentiation, apoptosis, and outgrowth of CNS cells during embryonic development. Several genes belonging to the Wnt/β-catenin cascade have been genetically associated with ASD.[91,92] Moreover, the mood-stabilizing drug lithium inhibits GSK-3, mimicking the activation of the Wnt/β-catenin signaling pathway.[93] In addition, Wnt/β-catenin signaling plays a prominent role in the regulation of excitatory synaptic transmission in pre- and postsynaptic compartments, thus contributing to E/I balance. At presynaptic level, Wnt/β-catenin signaling modulates cell adhesion, clustering, and recycling of synaptic vesicles.[94] Abnormalities in the presynaptic Wnt/β-catenin signaling translate in defects in spine morphogenesis and excitatory synaptic transmission.[95] At postsynaptic terminals, the Wnt/β-catenin pathway is involved in Ca^{2+} homeostasis through activation of different proteins such as L-type voltage sensitive Ca^{2+} channels, NMDA receptors, and CAMKII kinases.[96] This translates in a major role of Wnt/β-catenin pathway in the establishment of LTP and, therefore, the modulation of this signaling pathway through GSK-3β isoform could rescue defects in LTP and contribute to the fine-tuning of synaptic plasticity.

GSK-3β is constitutively active, and different upstream signaling cascades converge on GSK-3β to inhibit its activity. Studies with transgenic mice indicate that postnatal ablation of GSK-3β in the forebrain induces anxiolytic and prosocial effects,[97] whereas GSK-3β overexpression accounts for learning deficits.[98]

Although lithium has been widely used to manage mood disorders symptoms in psychiatric disorders, only a few studies have documented the effects of lithium administration in ASD patients. For instance, lithium administration to 30 children and adolescents diagnosed with ASD through DSM-IV-TR criteria improved symptoms such as euphoria, mania, or paranoia on 43% of patients.[99]

Chronic administration of lithium to neonatal rats exhibiting ASD-like behaviors abolished symptoms and improved defects in neurogenesis and E/I balance.[100] In addition, chronic lithium treatment reversed the increase in cerebral protein synthesis and ameliorated the behavioral abnormalities commonly observed in Fmr1 knockout mice.[101] In line with these studies, pharmacological inhibition of GSK-3β, using the inhibitor **53** (SB216763, Figure 8), reverses the hippocampus-dependent learning deficits and rescues adult hippocampal neurogenesis in FMR1 knockout mice, suggesting that modulation of Wnt/β-catenin is crucial in reactivating synaptic plasticity, and these effects may account for the observed behavioral and learning improvements.[102] In addition, administration of lithium or GSK-3 inhibitor **54** (AR-A014418, Figure 8) reduced

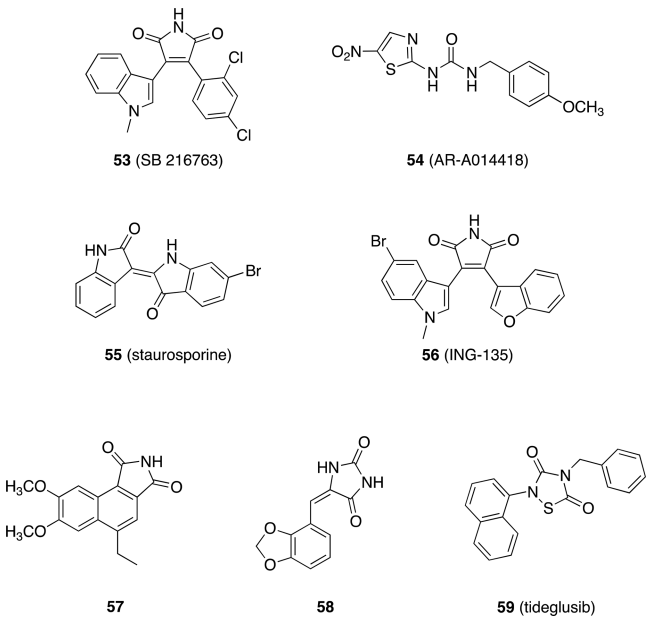

Figure 8. Structures of GSK-3β inhibitors.

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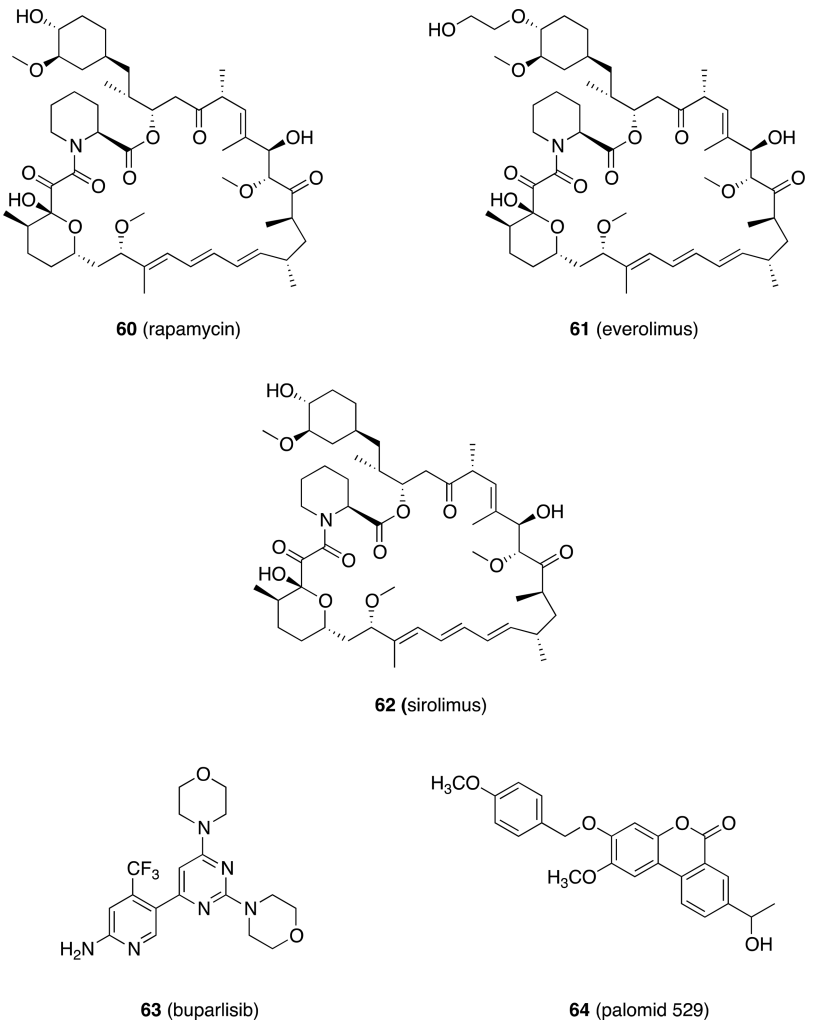

Figure 9. Structures of mTOR inhibitors.

audiogenic seizure susceptibility, a well-defined phenotype of FMFr1 knockout mice.[103] Finally, it has been proposed that the $mGlu_5$ NAM **20** selectively increases inhibitory GSK-3β phosphorylation in FMF1 knockout mice, mimicking the effect elicited by chronic lithium treatment.[104]

Numerous chemical scaffolds have been reported as small-molecule GSK-3β inhibitors mostly acting by competing with the ATP binding site of the kinase, while there are also known inhibitors which do not target the ATP binding site.[105] The first generation of ATP competitive inhibitors, such as compound **55** (staurosporine), lack suitable selectivity over other kinases. 3-(7-Azaindolyl)-4-arylmaleimides, exemplified by **53** and **56** (ING-135, Figure 8), benzo[*e*]isoindole-1,3-diones (compound **57**, Figure 8), and phenylmethylenehydantoins (compound **58**, Figure 8) belong to the second generation of GSK-3β endowed with good selectivity. 5-Imino-1,2,4-thiadiazole derivatives, exemplified by **59** (tideglusib, Figure 8) are reversible non-ATP competitive and substrate competitive inhibitors of GSK-3β.[105] Compound **59** is currently in phase II clinical trials for the treatment of adolescents with ASD (NCT02586935).

3.1.2. Dendritic Spine Morphology. In recent years, it has become evident that many psychiatric and neurologic disorders, including ASD, are linked to alterations in synapse structure and function and in dendritic spine morphology.[106] In addition, many of the genes associated with ASD encode proteins involved in synaptic transmission.[107] Dendritic spines are small membrane protrusions that contain the postsynaptic machinery, including glutamate receptors and the postsynaptic density (PSD) components, and contribute to the transmission of electrical signals. It is becoming increasingly apparent that synapse function and spine morphology are intimately linked. In fact, smaller spines have smaller synapses and this translates into reduced synaptic transmission. Abnormalities in spine density and morphology have been evidenced in ASD patients. A study on post-mortem ASD human brains has revealed an increase in spine density on apical dendrites of pyramidal neurons in frontal, temporal, and parietal lobes.[108] The observed increased spine density was inversely correlated with cognitive function. These findings are in line with the emerging hypothesis that the brains of ASD patients are characterized by hyperconnectivity in local circuits and hypoconnectivity between brain regions.[109] Spine dysgenesis is a common feature of syndromic forms of ASD. As an example, in FXS patients, spine morphology has been described as "immature" with long and tortuous spines, whereas in RTT individuals lower spine density and decreased proportion of mushroom-type spines in the cortex and hippocampus have been described.[36,40] In TSC mouse models it was observed a deficit in spinogenesis in early of postnatal life followed by impaired spine pruning, which led to higher spine densities in one-month-old mice.[42] It is therefore conceivable that converging deregulated signaling pathways downstream of the dysfunc-

DOI: 10.1021/acs.jmedchem.7b00965
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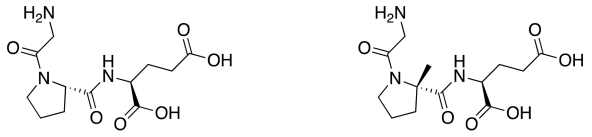
tional genes and upstream of dendritic spine formation and maturation exist.

PI3K/mTOR Pathway. PI3K/mTOR pathway regulates protein translation in dendrites near excitatory synapses and is being studied as a convergence point in syndromic forms of ASD. In FXS, FMRP is a regulator of protein translation in dendritic spines and modulates components directly downstream of the PI3K/mTOR pathway. In TSC, loss-of-function mutations in TSC1 and TSC2 proteins result in higher activity of mTOR. In MeCP2-deficient mice, a mouse model of RTT, the levels of mGlu receptors and BDNF, which are upstream in PI3K/mTOR pathways, are lower and this causes a decrease in levels of the serine/threonine kinase Akt and mTOR. In addition, mTOR activity increases dendritic protein translation, whereas in TSC, heightened mTOR activity impedes the synthesis of proteins required for stabilization of mGluR-dependent Ltd.[110] Therefore, it has been proposed that the level of mTOR activity should be within an appropriate range in order to support synaptic plasticity. In support of this hypothesis TSC and RTT, which are characterized by impaired mTOR-dependent protein translation, show impaired LTD, while FXS, which is characterized by sustained dendritic protein translation, displays elevated LTD.[111] In addition, mTOR inhibitors such as compounds **60** (rapamycin) and **61** (everolimus) (Figure 9) have been used to treat behavioral and molecular abnormalities in TSC deficient mice.[112] The positive results in preclinical studies mentioned above opened the way for randomized placebo-controlled trials of mTOR inhibitors for neurocognitive deficits in children with TSC (NCT01730209, NCT01289912, NCT01954693). A case report has described a 27 years-old female patient with TSC, autism, and renal angiomyolipomas, in whom treatment with compound **61** was associated with improvements in irritability, stereotypic behavior, and inappropriate speech.[113]

Abnormal mTOR activation has also been found in other neurodevelopmental disorders characterized by defective synaptogenesis or connectivity,[114] but the observed link could be epistatic or be caused by downstream effects of the defective gene rather than related to mTOR signaling per se.[115] In BTBR mice, compound **60** improved several measures of sociability (but not stereotypic behaviors), suggesting that mTOR overactivation represents a therapeutic target that mediates or contributes to impaired sociability in this mouse model of ASD.[116] One recent study has also implicated mTOR in ASD in humans: post-mortem analysis of brain tissue from ASD patients revealed increased density of dendritic spines on layer V pyramidal cells as well as aberrant mTOR activation and reduced autophagy.[117] However, whether mTOR is a key pathway in all types of autism or only in syndromic forms remains to be established. Moreover, translating the findings from preclinical models to humans will be challenging because the optimal temporal frame of the treatment is not known.[115] Finally, it has been reported that withdrawal of mTOR inhibitors leads to recrudescence of clinical symptoms.[118] In addition, because mTOR inhibitors might display potentially serious adverse effects, such as immunosuppression, mucositis, hyperlipidaemia, and dysmenorrhea, long-term treatment could be problematic.

To date, different chemical classes of PI3K/mTOR inhibitors have been reported in the literature, and numerous compounds have been advanced to clinical trials to treat brain cancer and even approved by the FDA. However, none of them has been studied in preclinical or clinical models of ASD, except **60** and **61**. BBB penetration is one of the main issues in the development of mTOR inhibitors because of unfavorable physicochemical properties or strong interaction with BBB efflux systems, as for compound **61** and the analogue **62** (sirolimus) (Figure 9). Compound **63** (buparlisib) and **64** (pamolid 529) (Figure 9) are two examples of mTOR inhibitors with favorable PK properties that are able to accumulate into the brain and to inhibit mTOR activity. Another approch to modulate PI3K/mTOR activity is the inhibition of the upstream Akt kinase. Also in this case, although different inhibitors have been reported, the main issue in the optimization is the brain penetrance.[119]

Insulin-Like Growth Factor-1. Insulin-like growth factor 1 (IGF-1) is a peptide hormone belonging to a superfamily of hormones termed insulin-like peptides. IGF-1 is synthesized and secreted by the liver in response to the growth hormone and acts on a broad range of cell types. IGF-1 is also produced in the CNS where it has a crucial role in growth, development, and maturation of both neuronal and glial cells and neuronal plasticity.[120] In the CNS, IGF-1 exerts its action by binding to IGF-1 receptor (IGF1R), a heterotetrameric glycoprotein expressed in both neural stem cells and all neural cells throughout the lifespan. When IGF-1 binds to IGF1R, the tyrosine kinase domains on the β subunits activate PI3K/Akt kinase and Ras-Raf-MAP pathways to induce downstream effects. Relevant downstream effectors of PI3K/Akt pathway are mTOR, GSK-3β, and β-catenin. Downstream effectors of Ras-Raf-MAP pathway are Erk1/2 and p38 MAPK, which are important in cellular maturation and survival (see ref 121 and references therein cited). During CNS development, IGF-1 is crucial for the differentiation and proliferation of neuronal cells as well as for their structural and functional integration into pre-existing neural circuitry, regulating changes in morphology, synaptic efficacy, and cellular organization. These effects are mediated by a myriad of mechanisms that include modulation of glutamatergic receptor units, alteration in Ca^{2+} channels conductance, modulation of E/I balance in neural circuitry, effects on synaptic proteins, and interactions with other neurotrophic factors.[121] As for the effect on spine morphology, Igf1−/− knockout mice display shorter dendrites, reducing dendritic spine density.[122] Once released in the serum, IGF-1 is cleaved into the active amino terminal glycine−proline−glutamate (GPE) tripeptide **65** ((1−3) IGF-1, Figure 10)



65 ((1-3) IGF-1, GPE) **66** (NNZ-2566)

Figure 10. Structures of compounds **65** and **66**.

and the truncated IGF-1 form called *des-N*-(1−3) IGF-1. Compound **65** is capable of crossing the BBB and retains strong neurotrophic and behavior-modifying activities.[123] Compound **65** displays neuroprotective effects as well as effects on excitatory synaptic markers, recapitulating many of the effects of IGF-1 on synaptic maturation and plasticity.[124] The effects of compound **65** may be different in neuronal and non-neuronal cell populations. Corvin et al. demonstrated that compound **65**, differently from IGF-1, is able to activate PI3K in glial cells and this reflects on synaptic markers because glial

DOI: 10.1021/acs.jmedchem.7b00965
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cells play a role in the formation and maintenance of synapses.[124] In addition, the same study proposed that compound **65** may indirectly activate the IGF-1 receptor by increasing the release of endogenous IGF-1.[124]

The therapeutic potential of IGF-1 and compound **65** in neurodevelopmental disorders such as RTT, FXS, as well as idiopathic ASD has been explored. Administration of compound **65** in Mecp2 mutant mice (a mouse model of RTT) led to an increase in brain size and excitatory synaptic markers, indicating that compound **65** promotes synapse maturation and influences synaptic plasticity. An increase of dendritic spine formation was also observed, and electrophysiology studies showed increased excitatory synaptic transmission in the sensorimotor cortex.[125] Moreover, administration of both IGF-1 and compound **65** in Mecp2 mice was able to improve social behavior.[126]

Deacon et al. have demonstrated that prolonged administration of compound **66** (NNZ-2566, Figure 10), an analogue of **65**, in FMR1 knockout mice was able to rescue of MAPK/ERK and PI3K/mTOR signaling abnormalities and to correct dendritic spine morphology. In addition, compound **66** in FMR1 knockout mice reduced anxiety levels and hyperactivity, improved short-term and long-term memory and learning, and normalized social recognition and behaviors.[127] The lack of adverse events and positive therapeutic profile in preclinical studies with FMR1 knockout mice provided evidence of the potential therapeutic effects of IGF-1 in FXS. A phase 2 industry-led clinical trial has been completed using compound **66**, with clinical improvement in many of the core symptoms in FXS patients (NCT01894958).

The studies illustrated above make IGF-1 a potentially attractive target for the treatment of ASD. In a study using neural cells derived from idiopathic ASD individuals, it was shown a partial rescue of deficits in neuronal networks (neuronal spike number and activity) on application of IGF-1.[128] At present, clinical trials are ongoing with the aim to use IGF-1 to treat the core symptoms of ASD (NCT01970345).

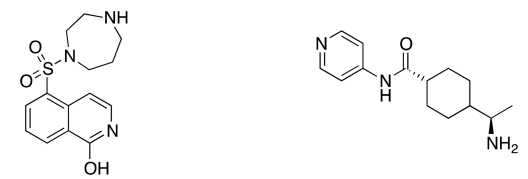
SHANK Proteins. Cumulative gene analysis in ASD subjects have identified several mutations in *SHANK3* gene, suggesting that abnormalities in this gene could be related to the neuropathology of ASD.[129] Moreover, mutations in *SHANK3* is a causable gene of Phelan−McDermid syndrome (PMDS) that is characterized by severe speech and expressive language deficits, global developmental delay, and autistic behavior.[130]

SH3 domain and ankyrin repeat containing protein (SHANK) proteins are major scaffolding proteins and have a major role in neuronal development. The SHANK family comprises three members: SHANK1, SHANK2, and SHANK3 proteins. SHANK3 is mainly localized in the PSD and is involved in cytoskeleton-associated signaling complex.[131] *SHANK3* gene encodes a multidomain protein containing ankyrin repeats, SH3 domain, PDZ domain, a proline-rich region, and the sterile alpha motif (SAM) domain.[132] Through these domains, SHANK3 can bind and interact with a wide variety of proteins, including modulators of small GTPases, such as RhoA and Cdc42, actin binding proteins, and actin modulators.[131] Dysregulation of SHANK proteins alters actin dynamics, and this translates in alterations in dendritic spine morphology and synaptic activity. Moreover, dysregulation of SHANK proteins leads to alteration in NMDA and AMPA receptors trafficking and, consequently, to alteration in the balance between E/I signals.[131]

It has been hypothesized that restoration of synaptic dysfuntion caused by abnormality of *SHANK3* gene may serve as a useful therapeutic strategy for ASD. IGF-1 has been proposed as a candidate molecule to restore synaptic dysfunctions related to *SHANK3* abnormalities. Daily intraperitoneal injections of IGF-1 for 2 weeks in *SHANK3*-deficient mice reversed deficits in hippocampal LTP, AMPA signaling, and motor performance.[133] Moreover, treatment of neurons differentiated from induced pluripotent stem cells (iPSC) from PMDS patients promotes the formation of mature excitatory synapses by increasing AMPA and NMDA receptors and corrects defects in excitatory synaptic transmission.[134] In a double blind, placebo controlled phase 2 trial, IGF-1 treatment significantly improved social impairment and restrictive behaviors.[135]

It has been proposed that enhancement of glutamate receptor activity may be of therapeutic relevance for the treatment of ASD related to alterations in SHANK proteins. In fact, SHANK3 is essential in mediating $mGlu_5$ receptor signaling by recruiting the scaffolding protein Homer1b/c to the PSD in the striatum and cortex.[136] A $mGlu_5$ PAM should enhance NMDA receptor function via $mGlu_5$ activation, and this would translate in improvement of synaptic plasticity. To this end, the $mGlu_5$ PAM **28** (Figure 3) was able to rescue behavioral deficits in *SHANK3* knockout mice.[137]

ROCK Kinases. The Rho family of GTPases is a family of small signaling G proteins belonging to the Ras superfamily. RhoA, Cdc42, and Rac1 belong to the Rho family of GTPases and are currently studied because they are regulators of actin dynamics and greatly influence dendritic spine biology and synaptic plasticity.[138] Active, GTPbound RhoA is a potent inhibitor of spine outgrowth through its main downstream effectors, Rho-associated coiled-coil containing protein kinases (ROCK) 1 and ROCK2, which are ubiquitous serine/threonine kinases.[139] Inhibition of both ROCK1 and ROCK2 with compound **67** (hydroxyfasudil, Figure 11) improved learning



67 (hydroxyfasudil) **68** (Y-27632)

Figure 11. Structure of the ROCK inhibitors that can modulate dendritic spines morphology.

and working memory in aged rats.[140] At the cellular level, ROCK1 and ROCK2 inhibition by compound **68** (Y-27632, Figure 11) increases the number and the proportion of thin spines, which are considered as precursors of mature spine. These results suggested that ROCK inhibition may enhance the capacity for synapse formation and structural plasticity in hippocampal neurons[141] and may be used to treat those CNS disorders characterized by altered dendritic spine morphology, such as ASD. It has also been proposed that dual inhibitors of ROCK1 and NADPH oxidase might be used to treat neurological diseases, including ASD.[142] To date, a large number of nonselective ROCK1/2 inhibitors have been reported (for a review see ref 143) and numerous compounds have entered in clinical trials for ophthalmic applications. Only a small number of nonselective ROCK1/2 inhibitors has

DOI: 10.1021/acs.jmedchem.7b00965
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entered clinical development for other applications, including CNS disorders, because of their narrow therapeutic window. In fact, ROCK1 inhibition has been related to cardiovascular adverse effects. Thus, selective ROCK2 inhibitors have been proposed as potential drugs to treat CNS disorders.[143] To date, selective ROCK2 inhibitors have not been described in the literature.

3.2. Targeting Central Neurotransmission Systems.

3.2.1. Serotonin System. The serotonin (5-hydroxytryptamine, 5-HT) system is involved in many neurobiological processes, including brain development. Disturbances in 5-HT neurotransmission have been indicated as an underlying cause of several neuropsychiatric disorders including ASD. Platelet hyperserotonemia was one of the first biochemical changes observed in ASD individuals, with 50−70% increase of the level of 5-HT in platelet compared to the normal value demonstrated in ∼30% of patients.[144] Variants in genes involved in the 5-HT system (the serotonin transporter gene *SLC6A4*) or in its degradation (the monoamine oxidase A gene) have been proposed to be related to ASD in humans.[145,146] In support of this, mice with mutations in the above genes show abnormal serotonergic transmission and social deficits.[147,148] In addition, developmental manipulations targeting 5-HT signaling in mice have indicated that excess 5-HT clearance during early stages of neurodevelopment could influence neuronal migration, axonal projections, and synapse development (see ref 149 and references therein cited). Thus, it is reasonable that defects in 5-HT system would affect circuits relevant for ASD-related behaviors.

As already illustrated above, SSRIs are used to treat depression, anxiety, and obsessive-compulsive behaviors in ASD individuals. However, the treatment with an SSRI has not shown to improve the core features of ASD nor other noncore aspects such as self-injurious behavior.[150] In contrast, a study in adults showed that compound **3** (Figure 1) gave significantly greater improvement in repetitive behaviors than placebo.[151] It is likely that the variability of SSRIs responses is a result of dysfunction of the 5-HT system at distinct levels (receptor, transport, processing, etc.).[149] Thus, the possibility of developing drugs acting on 5-HT system at more specific levels is being explored.

A clinical trial has determined the efficacy of the 5-HT_{1A} receptor partial agonist **69** (buspirone, Figure 12) on core symptoms and associated features in young children with ASD. Analyses of the main outcome of the study indicated that treatment with compound **69** did not result in decreased overall symptoms of ASD. On the other hand, secondary outcome measures demonstrated significant improvement in repetitive and restricted behaviors. The authors suggested that this treatment might be considered as an adjunct therapy to treat restrictive and repetitive behavior in association with early behavioral intervention.[152]

BTBR mice show reduced SERT density in various brain regions and increased 5-HT_{1A} receptor activity in the hippocampus.[153] In these mice, compounds **3** and **69** enhanced social interactions. Compound **1**, which is a dopamine D_2/5-HT_2 receptor antagonist, reduced marble burying, but had no effect on sociability in BTBR mice.[154] Also, in wild- type mice, drugs targeting 5-HT receptors, particularly 5-HT_{1A} and 5-HT_{2A} receptors, have shown promise for increasing social interaction or decreasing cognitive rigidity, which are behavioral phenotypes relevant to ASD.[155,156]

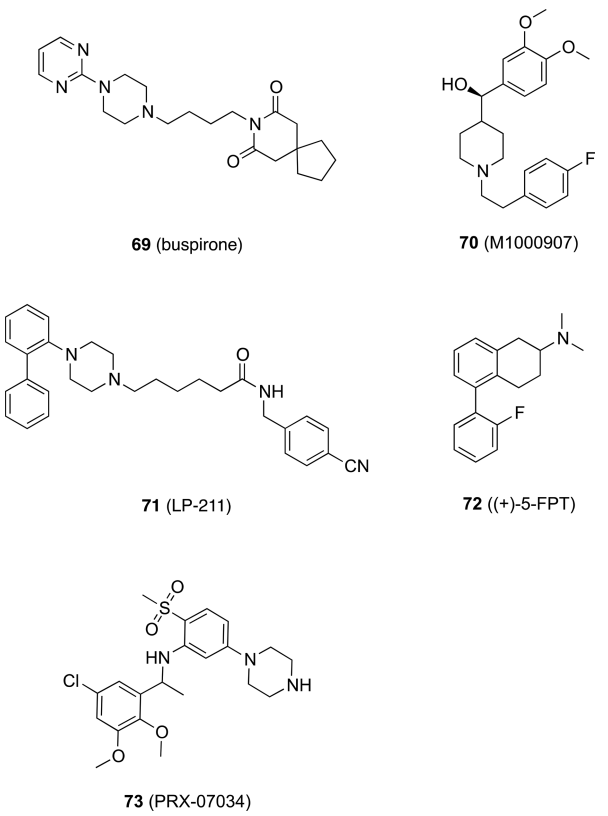

Figure 12. Serotonergic drugs and ligands that have been studied in ASD.

Preclinical and clinical studies have suggested that altered 5-HT_{2A} receptor signaling contributes to ASD symptoms.[147,149] To this end, systemic administration of the selective 5-HT_{2A} receptor antagonist **70** (M100907, Figure 12) in BTBR mice facilitates set-shifting and alleviates both a reversal learning deficit and elevated grooming behavior, suggesting that increased 5-HT_{2A} receptor activity in certain brain regions may contribute to repetitive behaviors of these mice.[157,158] Moreover, because compound **1** can cause unwanted side effects due to dopamine D_2 antagonism, treatment with a selective 5-HT_{2A} receptor antagonist has been proposed to improve cognitive flexibility in individuals with ASD.[157] In addition, microinfusion of **70** into the dorsomedial striatum alleviated a reversal learning impairment and attenuated grooming behavior, whereas the microinfusion into the orbitofrontal cortex increased perseveration during reversal learning and potentiated grooming. Consequently, it was suggested that elevated 5-HT_{2A} receptor activity in the dorsomedial striatum may contribute to behavioral inflexibility and stereotyped behaviors of BTBR mice. It was therefore suggested that systemic treatment with **70** principally acts on dorsomedial striatum to attenuate repetitive behaviors at least in BTBR mice.[159]

The involvement of 5-HT_7 receptors during brain development has emerged recently. It was demonstrated in mouse hippocampal neurons that activation of 5-HT_7 receptors stimulated the small GTP-ases RhoA and Cdc42 and enhanced neurite elongation, dendritic spine density, and the number of synaptic contacts. In addition, activation of 5-HT_7 receptors increased the expression of AMPA receptors and this led to increased synaptic efficacy.[160,161] Consistent with these data, stimulation of 5-HT_7 receptors by the agonist **71** (LP-211,

DOI: 10.1021/acs.jmedchem.7b00965
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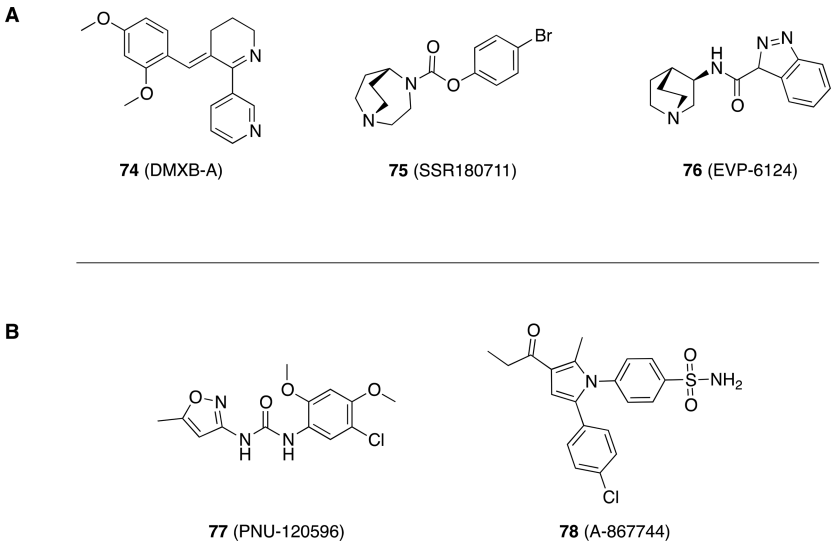

Figure 13. (A) Structures of α7 nACh agonists. (B) Structures of α7 nACh PAMs.

Figure 12) enhanced neurite outgrowth in embryonic neuronal primary cultures from hippocampus, cortex, and striatum by activating signaling transduction pathways that converge on the reorganization of cytoskeletal proteins.[162−164] These studies have proposed 5-HT$_7$ receptor as one of the key mediator of the well-known 5-HT effects in the correct establishment of neurites projections during critical periods of embryonic neuronal wiring. Very recently, it was also shown that the expression level of 5-HT$_7$ receptor in hippocampus progressively decreases during the postnatal development, wheres it is stable in cortex and striatum during the whole postnatal development.[165,166] Thus, it is very likely that the 5-HT$_7$ receptor participates in reorganization of neuronal networks and modulation of neural plasticity also during the later developmental stages and in adulthood. These data seem to be linked to the beneficial effects of 5-HT$_7$ receptor stimulation seen in mouse models of RTT and FXS. In a mouse model of RTT, 5-HT$_7$ receptor activation by compound **71** substantially rescues the neurobehavioral phenotype.[167−169] This effect may be linked to the capacity of 5-HT$_7$ receptor agonist to activate mTOR pathway. As for FXS, 5-HT$_7$ receptor activation reversed mGluR-mediated endocytosis of AMPA receptors and mGluR-LTD in both FMR1 knockout and wild-type mice.[170,171] In addition, systemic administration of the mixed 5-HT$_{1A}$ and 5-HT$_7$ receptor agonist **72** ((+)-5-FPT, Figure 12) reduced or abolished stereotypy in three different mouse models of stereotypy but not altered locomotor behavior on its own. Moreover, agonist **72** also enhanced social interaction.[172]

It has been suggested that blockade of 5-HT$_6$ receptors may be effective for individuals who suffer from working memory deficits such as in ASD because the selective 5-HT$_6$ receptor antagonist **73** (PRX-07034, Figure 12) was able to enhance working memory and cognitive flexibility in rats.[173] Interestingly, 5-HT$_6$ receptors are expressed early during brain development, and more direct evidence of their morphogenic role are being accumulated.[174,175]

5-HT receptor subtypes have long been objects of intense research with the aim to discover potent and selective agonists and antagonists. Over the last 30 years, hundreds of papers have been published on this topic. Several extensive reviews on the structure−activity relationships of 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_6$, and 5-HT$_7$ receptor agonists and antagonists may be recommended to those who have more interest in these topics.[176−180] While clinical candidates that could target selectively 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_6$ receptors are available, this is not the case for 5-HT$_7$ receptor. On the other hand, the studies illustrated above suggest that targeting more than one 5-HT receptor subtype could represent an approach to treat different behavioral features of ASD as, at least in part, shown by the study of Canal et al.[172] As targeting multiple 5-HT receptors has been pursued for other therapeutic purposes, the identification of serotonergic agents having an activity profile adequate to treat core symptoms of ASD can be envisaged.

3.2.2. Cholinergic System. Several post-mortem studies have evidenced abnormalities in the expression of cholinergic binding sites in brain areas of ASD individuals. In particular, the reduced expression of the gene encoding the $\alpha4\beta2$ nicotinic receptor in the cerebral cortex is a major feature of the neurochemical pathology of ASD, while post-transcriptional abnormalities of $\alpha4\beta2$ and $\alpha7$ nicotinic receptor subtypes are apparent in the cerebellum.[27] Moreover, cholinergic neurons project throughout the brain and contribute to the modulation of attention, learning, memory, cognitive flexibility, and sociability. Cholinergic projections are also critical for the maintenance of the E/I balance.[181] The evidence for cholinergic deficiencies has prompted the investigation of compound **16** (Figure 2) in animal models of ASD. Chronic administration of compound **16** in BTBR mice and in valproate-treated mice offspring improved cognitive flexibility in different behavioral paradigms, social behavior, and social recognition memory but did not induce any effect on repetitive behaviors. Initial outcomes of clinical trials with **15** and **17** (Figure 2) support further exploration of cholinergic intervention for the treatment of core and associated symptoms of ASD.[182]

It has been proposed that $\alpha7$ nicotinic acetylcholine receptor ($\alpha7$ nAChR) may be a valuable target in ASD. In fact, microdeletions in the proximal region of chromosome 15q between breakpoint (BP) 3 or BP4 and BP5 (15q13.3) encompassing *CHRNA7*, the gene encoding the $\alpha7$ nAChR, are associated with several neuropsychiatric disorders, including intellectual disability, schizophrenia, and ASD, suggesting that $\alpha7$ nAChR plays a crucial role in the developing brain and in the normal processes of attention, cognition, memory, and

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J. Med. Chem. XXXX, XXX, XXX−XXX

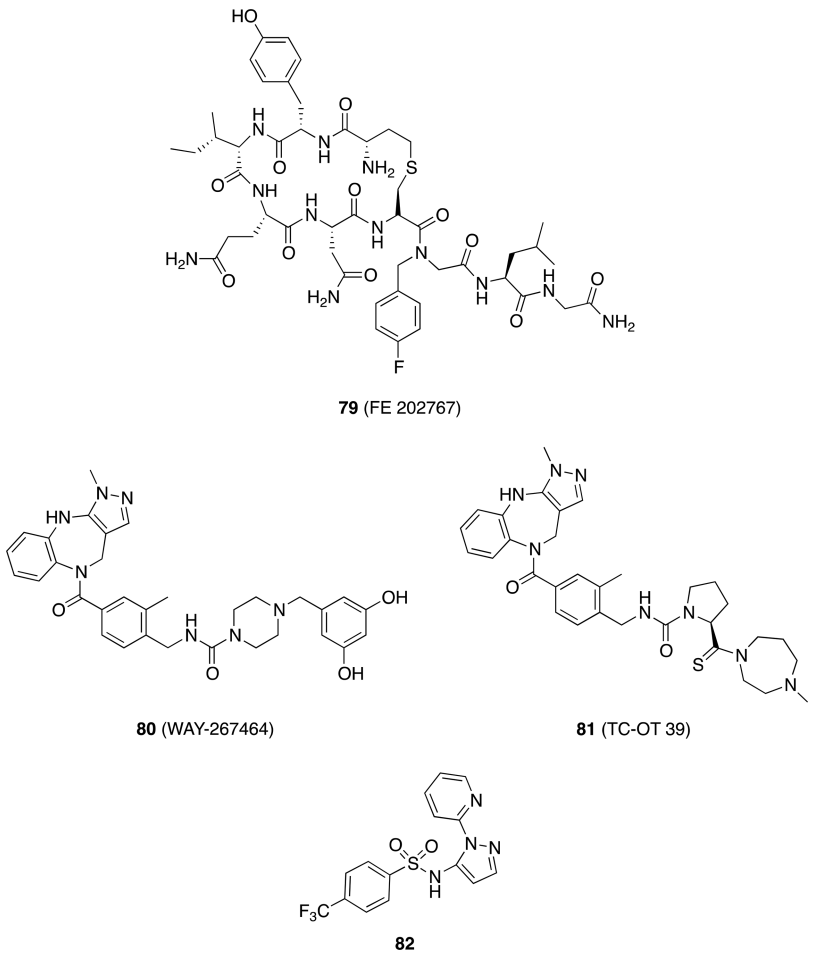

Figure 14. Peptidic and nonpeptidic oxytonergic ligands.

behavior throughout life.[183] The α7 nAChR is the most highly expressed nicotinic receptor subtype in the human brain, with particularly high levels in the cerebral cortex and the hippocampus. Within the hippocampus, α7 nAChRs facilitate the release of glutamate and GABA transmitters at the presynaptic level, whereas mediate fast synaptic transmission postsynaptically.[184]

Several drug discovery programs have targeted α7 nAChRs for neuropsychiatric disorders leading to the identification of brain penetrant full or partial agonists (for an extensive review see ref 185). Several α7 nAChR agonists, such as **74** (DMXB-A), **75** (SSR180711), and **76** (EVP-6124) (Figure 13), have shown to enhance cognitive functions and to elicit antipsychotic-like effects in preclinical models. However, no data have been reported yet in ASD animal models.[186]

It has also been proposed that the development of α7 nAChR PAMs may provide several advantages for therapeutics development. In fact, a key concern for α7 nAChR competitive agonists is the possibility that chronic administration may result in limited or diminished efficacy because of in vivo receptor desensitization and adverse effects due to the activation of other nicotinic receptor subtypes. Instead, PAMs require the endogenous ligand to elicit the activation of the nicotinic receptor and thus would maintain the natural temporal phasic stimulation pattern of the receptor. Several α7 nAChR PAMs have been developed, such as **77** (PNU120596)[187] and **78** (A-867744)[188] (Figure 13), and proved to be effective in preclinical models of cognitive and behavioral deficits associated with schizophrenia.[186] These results leave room for a potential application of α7 nAChR PAMs in ASD.

3.2.3. Oxytocin System. In recent years, oxytocin (OT) is receiving increased attention as a potential treatment for social deficits in ASD. OT is a hypothalamic neuropeptide linked to numerous social behaviors in mammals such as emotional bonding, maternal care, affiliation, and social attachment.[189,190] Altered OT concentrations have been reported in individuals with ASD, indicating that disturbances in OT levels lead to social and communicative dysfunction and suggesting that exogenous OT administration may be effective in reversing these symptoms.[191] It has been observed that ASD children have lower average levels of blood OT and higher OT precursors levels in comparison with typically developing age-matched children.[191,192] In addition, several genetic studies have suggested that ASD is linked to alteration of the genetic background of the OT receptor.[193] Finally, genetic variation in CD38, a transmembrane protein with ADP ribosyl cyclase activity involved in OT release, has been recently associated with differential response in social eye cues in children.[194]

Various randomized controlled trials of OT interventions in ASD patients have been carried out.[195] The outcomes of these studies demonstrate that, overall, OT is well tolerated and induces generally mild side effects either after intranasal or intravenous administration. The trials yielded potentially promising findings in neuropsychological measures of emotion recognition and eye gaze, whereas no significant improvements for repetitive behaviors were observed. The studies evidenced

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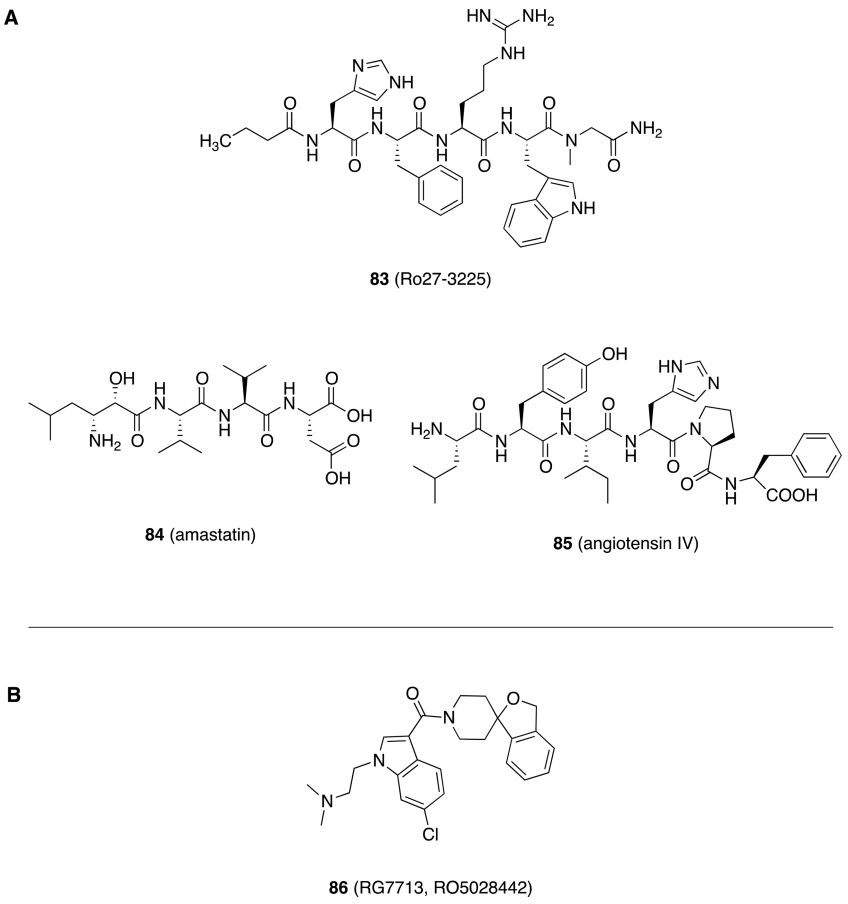

Figure 15. (A) Modulators of oxytonergic transmission. (B) Structure of the brain penetrant V1a antagonist **86**.

also a certain degree of variability among patients, although the effectiveness of OT was classified, in most of the cases, of medium size as compared to placebo. Only one study evidenced that the improvement in emotion recognition was maintained after 6 weeks of treatment with intranasal OT.

However, various concerns have been raised about the validity of these clinical trials, with respect to the unreliability of small clinical trials (median of the recruited patients was 15), questionable statistical analysis, and methodologic weaknesses.[196] In addition, only a very little fraction of the huge amounts of OT administered intranasally or intravenously reaches the cerebrospinal fluid and the brain. Instead, peripheral OT concentrations increase to supraphysiologic levels with likely side effects on peripheral organs.[196] Therefore, further studies are needed to establish OT as a treatment for individuals with ASD.

To circumvent the limitations of OT administration, efforts have been made to identify compounds with improved PK properties. Wisńiewski et al. have reported a series of potent and selective peptidic OT receptor agonists, exemplified by compound **79** (FE 202767, Figure 14), which shows excellent selectivity versus the related V_{1a}, V_{1b}, and V_2 vasopressin receptors and improved PK properties as compared to OT.[197] No data on behavioral efficacy have been reported.[197]

Nonpeptide small-molecule OT agonists or partial agonists could offer advantages over peptides because they can be designed to modulate potency, selectivity over the structurally similar vasopressin receptors, CNS penetration, and oral bioavailability.[198] Among nonpeptide agonists, compound **80** (WAY-267464, Figure 14) showed high affinity at both human and mouse OT receptors (K_i = 58.4 nM and 51.6 nM, respectively) and was characterized as an agonist in CHO-K1 cells stably expressing human or mouse OT receptor (EC_{50} = 61.3 and 29.0 nM, respectively). Moreover, compound **80** is 100-fold selective over V_{1a}, V_2, and V_{1b} vasopressin receptors.[199] The compound produces OT receptor-mediated anxiolytic effects in rodent behavioral paradigms similar to those elicited by OT. In the social preference test, compound **80** significantly improved social cognition.[199] Ferring Pharmaceuticals developed a series of potent nonpeptide agonists exemplified by compound **81** (TC-OT 39, Figure 14), which maximally stimulated OT receptor to the same extent as OT and was 25-fold selective over vasopressin V_2 receptors.[200] In a subsequent study, it was shown that compound **81** was only 2-fold selective over V_{1a} receptor.[201] Behavioral efficacy of compound **81** has not been reported. Hoffmann-La-Roche reported in a patent application a series of OT agonists with 5-sulfonamidopyrazole scaffold, exemplified by compound **82** (Figure 14). The compounds were specifically designed to treat CNS disorders related to OT dysfunction, but no data on either PK properties or behavioral efficacy of the compounds were disclosed.[202]

An alternative approach to modulate OT system is targeting receptors and pathways that affect OT levels. For instance, the neuropeptide galanin modulates OT release by acting on hypothalamo-neurohypophysal system.[203] The OT system can be also enhanced by inducing CD38 enzyme transcription. All-*trans*-retinoic acid (ATRA) is a potent inducer of CD38 and thus presumably of OT release. In a study on lymphoblastoid cell lines of ASD patients and their parents, ATRA increased

DOI: 10.1021/acs.jmedchem.7b00965
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CD38 mRNA expression, suggesting that molecules capable to induce CD38 transcription may be potential therapeutic candidates.[204]

Stimulation of melanocortin receptors located on oxytocinergic neurons of the supraoptic neurons is able to induce central, but not peripheral release of OT in rats. This effect can be blocked by the administration of a melanocortin 4 receptor (MC4R) antagonist.[205] The administration of compound **83** (Ro27-3225, Figure 15), a selective MC4R agonist in Cntnap knockout mice, restores social behavior.[206] Therefore, MC4R agonists could presumably increase central OT release, bypassing the limitations of peripheral peptide administration. The tone of OT system is also modulated by 5-HT. Serotoninergic neurons and receptors are localized on the oxytocinergic supraoptic nucleus and paraventricular nuclei of the hypothalamus, where they control the release of neurohypophyseal hormones.[207] Several papers have highlighted the complex interaction between the OT and 5-HT systems. As an example, 5-HT_{1A} receptor agonists, such as **69** or 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), substantially increase plasma OT levels and promote OT-dependent prosocial behaviors.[208,209] On the other hand, stimulation of 5-HT_{1B} receptor induces deficits in sociability in mice, preference for social novelty, and nonselective attention that can be reversed by administration of OT.[210]

These evidence indicate that there are potentially numerous druggable receptors on oxytocinergic neurons that could be targeted to increase OT release. This will require a systematic characterization to identify the target(s) leading to the greatest efficacy and the lowest off-target effects.

Finally, because extracellular OT is degraded by aminopeptidases, OT levels could be enhanced by limiting its enzymatic degradation. High levels of placental leucine aminopeptidase (P-LAP), which degrades OT, have been found in selected olfactory regions, hippocampus, and hypothalamus, co-localized with OT and vasopressin neurons, suggesting that P-LAP contributes to regulate OT levels in the brain.[211] Recently, competitive peptide P-LAP inhibitors **84** (amastatin) and **85** (angiotensin IV) (Figure 15) have been identified. As these molecules facilitate memory in different behavioral paradigms,[212] P-LAP inhibition may represent a viable strategy to enhance OT brain levels. However, aminopeptidases are not specific for OT and, therefore, potential side effects arising from the reduced degradation of other neuropeptides must be taken into account.

Vasopressin System. Besides OT, vasopressin has also been implicated in the regulation of social behavior in animals and humans.[213] Vasopressin interacts with three G-protein coupled receptors which are classified into V1 (V1a), V2, and V3 (V1b) receptor subtypes. In the rodent brain, vasopressin release increases during stress which causes passive coping behavior. This effect is reduced by the administration of peptidic V1 vasopressin receptor antagonists which, injected in the amygdala, reduce passive coping behavior.[214] A functional neuroimaging study in humans showed that vasopressin administration can modulate medial prefrontal cortex−amygdala circuitry during emotion processing.[215] In humans, V1a receptors are expressed in brain limbic areas and in cortical areas.[216] This pointed to a role of vasopressin in increasing the brain response to socially threatening stimuli in humans. It was postulated that V1a antagonists may have prosocial effects due to the modulation of the social brain, and therefore they may have potential for the treatment of psychiatric disorders related to social emotional dysfunction, including ASD.[217] Researchers at Roche, through an extensive medicinal chemistry campaign, identified the brain penetrant V1a antagonist **86** (RG7713 or RO5028442, Figure 15), devoid of V2 and OT receptors blocking properties.[218] A multicenter, randomized, double-blind study in adult male high-functioning ASD patients assessed the effects of **86** on behavioral and clinical measures of social cognition and communication. The results provided initial evidence that treatment with compound **86** provides subtle improvements in social communication surrogates in adults with high-functioning ASD, supporting further clinical exploration of V1a receptor antagonism as a therapeutic approach to treat core symptoms in ASD.[218]

3.3. Targeting Neuroinflammation. The presence of inflammation in ASD is a concept that is receiving momentum. Neuroinflammation is emerging as a common element in numerous neurological and neuropsychiatric disorders such as schizophrenia, bipolar disorder, and major depression.[219] This posed the question if neuroinflammation is a contributing mechanism in the development of ASD and if neuroinflammation is a causal or reactive process.

The hypothesis of a role of early (prenatal) inflammation in the etiology of ASD was initially based on the high correlation of ASD with the occurrence of viral epidemics evidenced by several epidemiological studies. Since then, maternal viral and bacterial infections and autoimmune diseases have been shown to be associated with the development of ASD. During brain development, cytokines and chemokines, which contribute to modulate neuronal and glial cell migration, differentiation, and synaptic maturation, are expressed at very low levels.[220] Maternal immune activation increases the levels in the maternal blood of specific brain chemokines and/or cytokines that could reach the fetal brain and affect brain development. As an example, increased concentration of IL-1β, IL-6, and TNF-α in the chord blood have been related to perinatal complications.[221,222] Additionally, it is not known how permeable is the developing BBB to antibodies and how maternal antibodies can reach fetal brain.[223]

Clinical and post-mortem studies show that in ASD inflammatory processes are not limited to the perinatal period. In fact, chronic inflammatory conditions and the abnormal response to infection have been described in ASD children and adults.[224]

Abnormal inflammatory processes may be an etiological factor in ASD that affects behavior and other symptoms throughout the life of ASD patients, as demonstrated by astrogliosis and microglial activation, along with increased expression of pro-inflammatory mediators, such as IL-6, TNF-α, TGF-β1, IFN-γ, IL-8, and other genes associated with the immune response in the brain and in the cerebrospinal fluid.[225]

Astrocyte pathophysiology can be critical in the progression of neurodevelopmental disorders. Astroglia organize the architecture of the brain, nurture synapses and perceive synaptic activity, participate in neurotransmission, neuron−astrocyte metabolic coupling, and cytokine secretion. As a result, astrocytes affect all processes associated with brain development, maturation, and aging.[226−228] Microglial cells are, on the other hand, the resident immune cells within the CNS that detect damage to the nervous system, secrete cytokines, and control neuroinflammation.[229] Thus, abnormal microglial activation or alteration of the physiological role of microglia in synapse removal may be crucial in neurodevelopmental disorders, including ASD.

DOI: 10.1021/acs.jmedchem.7b00965
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Different neuroinflammatory targets have been proposed for developing novel therapeutic strategies to target neuroinflammation in ASD.

In addition to the role in synaptic plasticity discussed above, $mGlu_5$ receptor is involved in the modulation of neurotrophic effects as well as proliferation and inflammatory responses of glial cells. $mGlu_5$ receptors are located on microglia where they are involved in neuronal−glial communication and contribute to the maintainance of neuronal homeostasis by controlling glutamate release and uptake by astrocytes.[230] It has been demonstrated that in primary microglia cultures the selective $mGlu_5$ receptor agonist (*RS*)-2-chloro-5- hydroxyphenylglycine (CHPG) can attenuate microglial activation as well as associated neurotoxicity following exposure to lipopolysaccharide (LPS).[230,231] Accordingly, studies in mouse models demonstrate that $mGlu_5$ receptor activation have neuroprotective effects by inhibiting microglial activation and proliferation.[230,232] Thus, there is evidence that $mGlu_5$ agonists may dampen down possible neuroinflammatory processes in ASD. However, considering the complex role of mGlu receptors in neuronal excitability and the cross-talk between metabotropic and ionotropic glutamate receptors, further studies are required to elucidate if chronic administration of selective $mGlu_5$ agonists may induce adverse effects, such as epileptic activity or seizures, and if $mGlu_5$ PAMs may also exert neuroprotective effects by inhibiting microglial activation.

It has been proposed that inflammasome activation through Toll-like receptor (TLR) 3- or TLR4-mediated pathways may underlie well-known animal models of ASD, such as LPS- or poly(I:C)- or group B streptococcus-induced maternal immune activation.[233−235] TLR3 and TLR4 are expressed in astrocytes and microglia, and it seems that the response of astrocytes to TLR3 and TLR4 agonists is dependent on the presence of functional microglia.[236] At the molecular level, TLR4 activation by the agonist LPS is related to the increased production of inflammatory cytokines/chemokines and ROS by activation of NFκB pathway.[234]

Modulation of TLR4 signaling pathway has been recently proposed as a new therapeutic approach to reduce the inflammatory burden in ASD children.[237] TLR4 signaling was explored in peripheral T cells of ASD patients. TLR4 expression was upregulated in ASD children as compared to normal controls. The activation of TLR4 signaling was associated with increased expression of NOX-2 and ROS generation, which was reduced by inhibition of NFκB pathway.[237] These findings are in agreement with previous studies reporting that stimulation of peripheral blood mononuclear cells of ASD children with LPS enhanced proinflammatory cytokine production and that NFκB expression was shown to be elevated in ASD patients in both peripheral blood and CNS.[238]

In recent years, the elucidation of the crystal structure of the complex formed by TLR4 and accessory proteins has strongly supported progresses in the design of small-molecule TLR4 antagonists. Several chemical classes of TLR4 antagonists have been described and studied in different models of peripheral or central inflammation.[239] Among these, the β-aminoalcohol derivatives **87** and **88** (Figure 16) have proved to be effective suppressor of TLR4 activation with good solubility and PK properties.[239] Compound **89** (TAK-242, Figure 16) suppresses TLR4 activation by blocking the formation of TLR4−accessory proteins complex and thus the activation of the downstream pathways related to the production of pro-inflammatory cytokines.[239] Compound **89** was able to decrease neuroinflammation in rat frontal cortex after stress.[240] No data in animal models of ASD have been reported to date. It has been also reported that tricyclic antidepressants, such as **90** (amitriptyline), **91** (mianserin), and **92** (desimipramine) (Figure 16), have showed varying degrees of inhibition on TLR4 activation in different cell lines, including murine microglial BV-2 cells.[241]

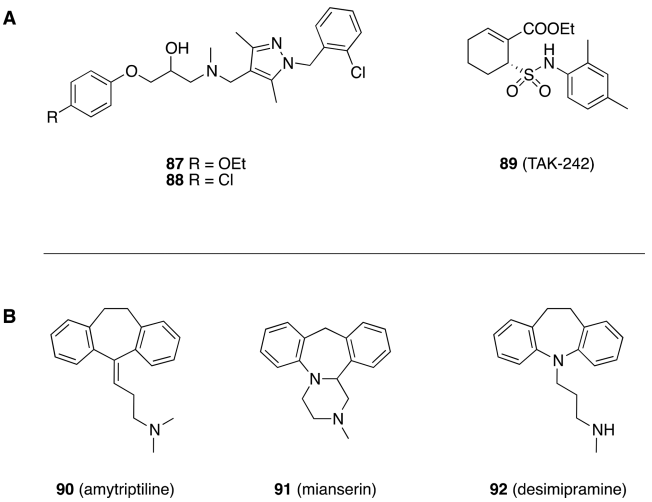

Figure 16. (A) Structures of TLR4 agonists. (B) Tricyclic antidepressants that can inhibit TLR4 activation.

Besides the role in the modulation of synaptic plasticity and OT release, 5-HT may also have a role in the modulation of neuroinflammation. Serotonin functions not only as a neurotrophic factor controlling brain development, but it also modulates immune response.[242] In fact, 5-HT modulates a wide variety of immune functions, such as inflammation, phagocytosis, T cell migration, and cytokine production.[243] To date, the intercommunication between serotonergic events and abnormalities of immune responses in autistic phenotypes has not been elucidated. High levels of 5-HT can directly influence innate and adaptive response of the immune system and influence overproduction of cytokines and chemokines, which, in turn, can enter the brain influencing neuronal maturation in the developmental stages. Increased reuptake results in reduced 5-HT levels, which are important for proper function of the immune system.[244] On the other hand, the increased expression of pro-inflammatory cytokines regulates SERT function through p38 mitogen activated protein kinase (MAPK) signaling pathway and induces behavioral changes.[245] It is therefore evident that a better understanding of the relation between serotonergic transmission and immune system can open new therapeutic perspectives to address behavioral deficits in ASD.

Starting from the observation that children with ASD show increased levels of neurotensin (NT) in the serum,[246] Patel and co-workers have reported that NT is able to activate primary microglia cultures obtained from human brains as well as the immortalized human microglial cell line SV40.[247] They showed that NT increases gene expression and release of the pro-inflammatory cytokine IL-1β and chemokines CXCL8, CCL2, and CCL5 by activating sortilin and not neurotensin receptors 1 or 2. Sortilin is a type I membrane receptor belonging to the vacuolar protein sorting 10 protein (VPS10P) family of sorting receptors[248] and is mainly expressed in CNS during embryonic

DOI: 10.1021/acs.jmedchem.7b00965
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development and inflammatory processes.[249,250] On such basis, it has been suggested that inhibiting sortilin may provide novel therapeutic approaches for ASD. However, challenging sortilin signaling could induce several adverse effects due to the important role of sortilin in numerous physiological functions in the body.[251] Researchers at Lundbeck have recently disclosed the orally bioavailable small-molecule sortilin inhibitor **93** (AF38469, Figure 17).[252] However, the compound exhibits

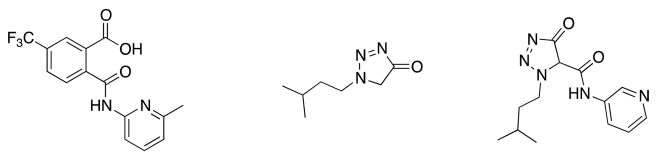

Figure 17. Structures of sortilin inhibitors.

poor CNS exposure because of the presence in the molecule of a carboxylic group, and hence it is not useful to study sortilin biology in CNS in vivo. In a subsequent study, using fragment-based and structure-based drug design approaches, *N*-substituted 1,2,3-triazol-4-one/ol was identified as a template for the development of sortilin inhibitors because it acts as a carboxylic acid isostere and allows crucial interactions with sortilin. Compounds **94** and **95** were identified as cell permeable sortilin inhibitors, albeit with modest potency (Figure 17).[253]

Matrix metalloproteinases (MMPs) is a group of proteases involved in neuroinflammation and neurodevelopment processes and, as such, they have been indicated as possible players in the etiopathology of ASD. For example, MMPs can either promote or suppress inflammation through proteolysis of cytokines and chemokines.[254] However, despite the biologic plausibility of the involvement of MMPs in ASD,[255] further investigations in this area are needed. Interestingly, it has been reported that FMR1 knockout mice show abnormal elevated expression of MMP-9 in the brain which can be downregulated by treatment with compound **96** (minocycline, Figure 18), a

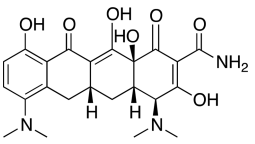

Figure 18. Structure of compound **96**.

tetracycline antibiotic with MMP-9 inhibitor activity. Down-regulation of MMP-9 rescues either immature dendritic spine morphology and abnormal behavior.[256] In addition, high plasma activity of MMP-9 was observed in FXS patients. Thus, subsequent trials showed that activity levels of MMP-9 could be lowered by administration of compound **96** and that, in some cases, changes in MMP-9 activity were positively associated with improvements of clinical measures.[257] These findings might be relevant also for ASD because elevations of MMP-9 in amniotic fluid samples in ASD cases have been reported.[255] Thus, MMP-9 inhibitors might be pursued for treatment of ASD. Several efforts have been done in order to develop selective inhibitors of MMPs, leading to the identification of different chemical classes of inhibitors. As for MMP-9 inhibitors, however, compound **96** is the only small molecule able to cross the BBB available to date.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

Over the past few years, more and more data have become available on the etiology of ASD. As the neurobiology underlying ASD is being discovered, targeted drug therapy is becoming possible, at least in theory. The very high clinical and etiological variability between individuals with ASD indicates that no single treatment will benefit every ASD patient. Identifying the genetic causes of ASD has proven to be elusive, as ASD is believed to be polygenic. However, the "many genes, common pathways" hypothesis suggests that the many genes associated with ASD will converge, through different molecular mechanisms, onto common processes in the brain responsible for the core symptoms of ASD.[258] The elucidation of these common pathways will likely lead to the development of therapies to treat core symptoms of ASD. However, to estimate whether a target may be useful for large patient groups it is important to understand how different etiologies converge on specific molecular mechanisms and how they map onto difference in circuit-level brain, cognitive development, and behavioral symptom profiles.

Animal models of syndromic and nonsyndromic forms of ASD have greatly advanced the understanding of the biochemical pathways involved in ASD and have provided the opportunity to study developmental changes or expression of genetic variants in different brain areas and to manipulate brain regions and circuits to test their precise functions. However, animal models fail to recapitulate many aspects of ASD due to species differences and to the possible contribution of epigenetics in the pathophysiology of ASD.[32] In addition, some neocortical regions affected in humans are not obtainable from mouse brain, and brain development of mice does not perfectly reflect the development of the human brain.[259] Therefore, clinical studies in human populations are crucial for understanding the genetic and nongenetic contributions to ASD and for validating potential drug targets. With this respect, iPSCs technology is providing a remarkable alternative tool to bridge the translational gap between animal models and human clinical trials for the study of human brain diseases through the scalable, manipulable production of human neural cells derived directly from ASD patients. Recent progress in iPSC technology as well as in the techniques for in vitro neural differentiation have allowed to functionally characterize neurons and to analyze cortical development during neural differentiation contributing to the understanding of the pathogenic mechanisms of ASD and to identify molecular biomarkers for patient stratification and personalized medicine.[259]

As illustrated in the present perspective, synaptic dysfunction is receiving much attention. Imbalance between excitatory and inhibitory transmission is a common mechanism in ASD that is responsible for learning and memory, cognitive, sensory, motor deficits, and seizures. E/I imbalance can be corrected by acting on mGlu, NMDA, or GABA receptors or by inhibiting GSK-3β signaling. Medicinal chemistry efforts have eventually led to the identification of molecules targeting mGlu, NMDA, and GABA receptors that have entered clinical trials. As for GSK-3β inhibitors, promising candidates have been identified. Developing highly selective allosteric modulators or targeting the intracellular pathways downstream of glutamatergic receptors

DOI: 10.1021/acs.jmedchem.7b00965
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may open new therapeutic perspectives because they can enhance neuroplasticity without inducing neurotoxicity.

Another strategy to correct synapse disfunction is targeting abnormalities in dendritic spine morphology and density. mTOR is probably the most studied and promising target, and the inhibitors **60** and **61** have entered clinical trials for treatment of ASD core symptoms. The outcomes of clinical trials will answer the question if targeting mTOR will translate into clinical improvements. ROCK1 and ROCK2 kinases have also been proposed as valuable target in this context but inhibitors targeting the brain have not been identified yet. However, it has been pointed out that challenging ubiquitous targets such as mTOR or ROCK kinases may be associated with potentially serious adverse side effects that can negatively impact on long-term tolerability and compliance.

Targeting IGF1 system is another strategy to correct dendritic spine abnormalities. Early clinical evidence obtained with compound **66** (Figure 10) are encouraging. However, how compound **66** exerts its effects is still not completely elucidated. Once this will be clarified, it will be possible to design new analogues of compounds **65** and **66** that will likely open new perspectives in drug development.

Besides glutamatergic and GABAergic transmission, the therapeutic potential of other neuronal transmission systems, such as OT, vasopressin, acetylcholine, and serotonin, have been explored in ASD field. OT has become a focus of investigation because of its role in social behavior and the ongoing clinical trials will contribute to assess the therapeutic potential of addressing oxytonergic system. However, the efficacy of long-term OT administration as well as potential detrimental effects related to overstimulation of this system are still to be fully elucidated. An alternative approach could be targeting receptors and pathways that modulate OT levels. In this respect, it has been proposed that targeting galanin or melanocortin receptors could deserve investigation. While small-molecule melanocortin agonists have been identified so far,[260] the same does not apply for galanin receptors.

Central serotonin neurotransmission may also be targeted for treatment of ASD. It has been shown that 5-HT_{1A} receptor agonists or 5-HT_{2A} receptor antagonists act on neural circuits relevant to ASD, whereas 5-HT_7 agonists or 5-HT_6 antagonists play a role in synaptic plasticity by acting on RhoGTPase, thus correcting abnormalities in dendritic spine morphology. While clinical candidates that could target selectively 5-HT_{1A}, 5-HT_{2A}, and 5-HT_6 receptors are available, this is not the case for 5-HT_7 receptor. In addition, it is likely that targeting multiple serotonin receptor subtypes can lead to the identification of new serotonergic agents having an activity profile adequate to treat core symptoms of ASD. Recent studies have proposed that acetylcholine neurotransmission may contribute to synaptic plasticity through the activation of $\alpha 7$ nicotinic receptor, which, in turn activates RhoGTPases leading to cytoskeletal changes during neurite growth.[261] These data might add a new target to correct abnormal synaptic plasticity.

Besides synaptic dysfuntion and defects in certain neurotransmitter systems, neuroinflammation is also thought to underlie ASD pathology. Current research is indicating Toll-like receptors, such as TLR3 and TLR4, MMP-9, and sortilin as possible players in neuroinflammation and it is likely that other players will be identified in the near future. Of note, targets initially investigated to correct synaptic dysfunction (i.e.: $mGlu_5$ receptor, IGF-1 signaling, and some 5-HT receptor subtypes) are also important players in neuroinflammation. With this respect, the study of the interaction between neurons, microglia, and astrocytes is at the very beginning. It is now becoming evident the impact of neuroinflammation on synapses functioning, and this could unveil new scenarios even more complex than present.

As already pointed out, the wide heterogeneity of clinical and behavioral symptoms in ASD suggests that no single treatment will be efficacious to treat every ASD patient. The assessment of the validity of the target will greatly depend on the selection of patient groups for clinical trials that are likely to respond to the treatment under consideration. This requires the identification and validation of stratification biomarkers that divide patients into subgroups with shared biological characteristics.[262] To date, no generally accepted biomarkers for ASD diagnosis exist. In addition, biomarkers will supplement the outcomes of clinical trials.

Another crucial point to assessing the validity of an ASD drug target is the identification of the critical periods when different factors impact on neuronal development. Brain development is initially determined by distinct temporal and spatial stages of gene expression and intrinsic neuronal activity, then it is refined by interactions with the environment. Therefore, some treatment effects may be different in developing or adult brains. The vast majority of animal studies have been carried out only on adult animals, and this impacts on the translatability of the outcomes to humans.

Finally, a treatment that is likely to be only effective in early development would raise important implications for the design of clinical trials, which usually test efficacy and side effects in adults. The common phrase "children are not little adults" needs to be true even in the drug development stage. All these different factors will converge to address the challenges posed by ASD through a personalized medicine approach.

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Notes

The authors declare no competing financial interest.

Biographies

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Roberto Perrone is Full Professor of Medicinal Chemistry in the Department of Pharmacy—Drug Science at the University of Bari Aldo Moro since 1991. Since 1987, he has been teaching Pharmaceutical Chemistry for graduate courses of Pharmacy and Chemistry and Pharmaceutical Technologies at the University of Bari. Since 2007, he has been elected Director of the Department of

DOI: 10.1021/acs.jmedchem.7b00965
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Lucia Margari obtained a degree in Medicine and Surgery at the University of Bari in 1978. Later, she completed her training to become a Specialist in Neurology (1983) and in Child and Adolescent Neuropsychiatry (1997) at the University of Bari Aldo Moro. Since 2006, she is Full Professor of Child and Adolescent Neuropsychiatry at the University of Bari Aldo Moro. She is Director of the School of Specialization in Child and Adolescent Neuropsychiatry at the same University since 2004 and Director of the Unit of Child Neuropsychiatry at the Policlinico Hospital of Bari since 2009. She is author of more than 170 scientific publications on national and international journals, monographs, book chapters, and conference proceedings.

Marcello Leopoldo obtained the degree in Chemistry and Pharmaceutical Technologies (honors) at the University of Bari in 1991. At the same University, he was appointed Research Associate in Medicinal Chemistry (1992) and Associate Professor in Medicinal Chemistry in 2001 (Department of Pharmacy—Drug Sciences). He has received the Habilitation as Full Professor in Medicinal Chemistry in 2015. He has published more than 100 papers on peer-reviewed journals, focused on the development of selective ligands for dopamine and serotonin receptors. In particular, the potent and selective 5-HT_7 receptor agonist LP-211 was identified in his lab nearly 10 years ago. LP-211 is being used to explore the potential therapeutic effects of 5-HT_7 receptor activation in neurodevelopmental disorders. He is inventor of eight patent applications.

■ ACKNOWLEDGMENTS

We thank the Rector of the University of Bari Aldo Moro, Professor Antonio Felice Uricchio, who established the Working Group on Autism (Gruppo di Lavoro per l'Autismo).

■ ABBREVIATIONS USED

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASD, autism spectrum disorder; BBB, blood–brain barrier; CDPPB, 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide; CNS, central nervous system; FXS, fragile X syndrome; GABA, gamma aminobutyric acid; LTD, long-term depression; LTP, long-term potentiation; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; MTEP, 3-((2-methyl-4-thiazolyl)ethynyl)pyridine; mTOR, mammalian target of rapamycin; NAM, negative allosteric modulator; NCFP, *N*-(4-chloro-2-((4-fluoro-1,3-dioxoisoindolin-2-yl)methyl)phenyl)picolinamide; NMDA, *N*-methyl-D-aspartate; OCD, obsessive–compulsive disorder; OT, oxytocin; PAM, positive allosteric modulator; PK, pharmacokinetic; PSD, postsynaptic density; RTT, Rett syndrome; SSRI, selective serotonin reuptake inhibitors; TSC, tuberous sclerosis complex

■ REFERENCES

(1) *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed.; American Psychiatric Association: Washington, DC, 2013.

(2) Gotham, K.; Risi, S.; Pickles, A.; Lord, C. The autism diagnostic observation schedule: revised algorithms for improved diagnostic validity. *J. Autism Dev. Disord.* **2007**, *37*, 613–627.

(3) Rutter, M.; Le Couter, A.; Lord, C. *ADI-R, Autism Diagnostic Interview: Revised*. Western Psychological Services, Los Angeles, CA, 2003.

(4) Maski, K. P.; Jeste, S. S.; Spence, S. J. Common neurological comorbidities in autism spectrum disorders. *Curr. Opin. Pediatr.* **2011**, *23*, 609–615.

(5) Leyfer, O. T.; Folstein, S. E.; Bacalman, S.; Davis, N. O.; Dinh, E.; Morgan, J.; Tager-Flusberg, H.; Lainhart, J. E. Comorbid psychiatric disorders in children with autism: interview development and rates of disorders. *J. Autism Dev. Disord.* **2006**, *36*, 849–861.

(6) Persico, A. M. Autisms. In *Neural Circuit Development and Function in the Healthy and Diseased Brain: Comprehensive Developmental Neuroscience*; Vol. 3; Rakic, P., Rubenstein, J., Eds.; Elsevier: New York, 2013; pp 651–694.

(7) Baio, J. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *MMWR Surveill. Summ.* **2014**, *63*, 1–21.

(8) Levy, S. E.; Mandell, D. S.; Schultz, R. T. Autism. *Lancet* **2009**, *374*, 1627–1638.

(9) DiCicco-Bloom, E.; Lord, C.; Zwaigenbaum, L.; Courchesne, E.; Dager, S. R.; Schmitz, C.; Schultz, R. T.; Crawley, J.; Young, L. J. The developmental neurobiology of autism spectrum disorder. *J. Neurosci.* **2006**, *26*, 6897–6906.

(10) Bauman, M. L.; Kemper, T. L. Neuroanatomic observations of the brain in autism: a review and future directions. *Int. J. Dev. Neurosci.* **2005**, *23*, 183–187.

(11) Rice, D.; Barone, S., Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ. Health Perspect.* **2000**, *108*, 511–533.

(12) Geschwind, D. H.; Levitt, P. Autism spectrum disorders: developmental disconnection syndromes. *Curr. Opin. Neurobiol.* **2007**, *17*, 103–111.

(13) Hahamy, A.; Behrmann, M.; Malach, R. The idiosyncratic brain: distortion of spontaneous connectivity patterns in autism spectrum disorder. *Nat. Neurosci.* **2015**, *18*, 302–309.

(14) Geschwind, D. H. Genetics of autism spectrum disorders. *Trends Cognit. Sci.* **2011**, *15*, 409–416.

(15) Persico, A. M.; Merelli, S. Environmental factors and autism spectrum disorder. *Curr. Dev. Disord. Rep.* **2014**, *1*, 8–19.

(16) Young, A. M.; Chakrabarti, B.; Roberts, D.; Lai, M. C.; Suckling, J.; Baron-Cohen, S. From molecules to neural morphology: understanding neuroinflammation in autism spectrum condition. *Mol. Autism* **2016**, *7*, 9.

(17) Ji, N.; Findling, R. L. An update on pharmacotherapy for autism spectrum disorder in children and adolescents. *Curr. Opin. Psychiatry* **2015**, *28*, 91–101.

(18) Matera, E.; Margari, L.; Palmieri, V. O.; Zagaria, G.; Palumbi, R.; Margari, F. Risperidone and cardiometabolic risk in children and adolescents: clinical and instrumental issues. *J. Clin. Psychopharmacol.* **2017**, *37*, 302–309.

(19) Margari, L.; Matera, E.; Petruzzelli, M. G.; Simone, M.; Lamanna, A. L.; Pastore, A.; Palmieri, V. O.; Margari, F. Prolactin variations during risperidone therapy in a sample of drug-naive children and adolescents. *Int. Clin. Psychopharmacol.* **2015**, *30*, 103–108.

(20) Margari, L.; Matera, E.; Craig, F.; Petruzzelli, M. G.; Palmieri, V. O.; Pastore, A.; Margari, F. Tolerability and safety profile of risperidone in a sample of children and adolescents. *Int. Clin. Psychopharmacol.* **2013**, *28*, 177–183.

(21) McDougle, C. J.; Stigler, K. A.; Erickson, C. A.; Posey, D. J. Atypical antipsychotics in children and adolescents with autistic and other pervasive developmental disorders. *J. Clin. Psychiatry* **2008**, *69* (Suppl 4), 15–20.

(22) McCracken, J. T.; McGough, J.; Shah, B.; Cronin, P.; Hong, D.; Aman, M. G.; Arnold, L. E.; Lindsay, R.; Nash, P.; Hollway, J.; McDougle, C. J.; Posey, D.; Swiezy, N.; Kohn, A.; Scahill, L.; Martin, A.; Koenig, K.; Volkmar, F.; Carroll, D.; Lancor, A.; Tierney, E.; Ghuman, J.; Gonzalez, N. M.; Grados, M.; Vitiello, B.; Ritz, L.; Davies, M.; Robinson, J.; McMahon, D. Research units on pediatric psychopharmacology autism network. Risperidone in children with

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX–XXX

autism and serious behavioral problems. *N. Engl. J. Med.* **2002**, *347*, 314−321.
(23) Moore, M. L.; Eichner, S. F.; Jones, J. R. Treating functional impairment of autism with selective serotonin-reuptake inhibitors. *Ann. Pharmacother.* **2004**, *38*, 1515−1519.
(24) Williams, K.; Brignell, A.; Randall, M.; Silove, N.; Hazell, P. Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Cochrane Database Syst. Rev.* **2013**, *8*, CD004677.
(25) Hurwitz, R.; Blackmore, R.; Hazell, P.; Williams, K.; Woolfenden, S. Tricyclic antidepressants for autism spectrum disorders (ASD) in children and adolescents. *Cochrane Database Syst. Rev.* **2012**, *3*, CD008372.
(26) Hirota, T.; Veenstra-Vanderweele, J.; Hollander, E.; Kishi, T. Antiepileptic medications in autism spectrum disorder: a systematic review and meta-analysis. *J. Autism Dev. Disord.* **2014**, *44*, 948−957.
(27) Perry, E. K.; Lee, M. L.; Martin-Ruiz, C. M.; Court, J. A.; Volsen, S. G.; Merrit, J.; Folly, E.; Iversen, P. E.; Bauman, M. L.; Perry, R. H.; Wenk, G. L. Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. *Am. J. Psychiatry* **2001**, *158*, 1058−1066.
(28) Rossignol, D. A.; Frye, R. E. The use of medications approved for Alzheimer's disease in autism spectrum disorder: a systematic review. *Front. Pediatr.* **2014**, *2*, 87.
(29) Pearson, D.; Santos, C.; Aman, M.; Arnold, L. E.; Casat, C. D.; Mansour, R.; Lane, D. M.; Loveland, K. A.; Bukstein, O. G.; Jerger, S. W.; Factor, P.; Vanwoerden, S.; Perez, E.; Cleveland, L. A. Effects of extended release methylphenidate treatment on ratings of attention-deficit/hyperactivity disorder (ADHD) and associated behavior in children with autism spectrum disorders and ADHD symptoms. *J. Child Adolesc. Psychopharmacol.* **2013**, *23*, 337−351.
(30) Panksepp, J.; Lensing, P. Brief report: a synopsis of an open-trial of naltrexone treatment of autism with four children. *J. Autism Dev. Disord.* **1991**, *21*, 243−249.
(31) Eichaar, G. M.; Maisch, N. M.; Augusto, L. M.; Wehring, H. J. Efficacy and safety of naltrexone use in pediatric patients with autistic disorder. *Ann. Pharmacother.* **2006**, *40*, 1086−1095.
(32) Varghese, M.; Keshav, N.; Jacot-Descombes, S.; Warda, T.; Wicinski, B.; Dickstein, D. L.; Harony-Nicolas, H.; De Rubeis, S.; Drapeau, E.; Buxbaum, J. D.; Hof, P. R. Autism spectrum disorder: neuropathology and animal models. *Acta Neuropathol.* **2017**, *134*, 537−566.
(33) Sztainberg, Y.; Zoghbi, H. Y. Lessons learned from studying syndromic autism spectrum disorders. *Nat. Neurosci.* **2016**, *19*, 1408−1417.
(34) Jacquemont, S.; Hagerman, R. J.; Hagerman, P. J.; Leehey, M. A. Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: two faces of FMR1. *Lancet Neurol.* **2007**, *6*, 45−55.
(35) Huber, K. M.; Gallagher, S. M.; Warren, S. T.; Bear, M. F. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 7746−7750.
(36) Irwin, S. A.; Galvez, R.; Greenough, W. T. Dendritic spine structural anomalies in fragile-X mental retardation syndrome. *Cereb. Cortex* **2000**, *10*, 1038−1044.
(37) Bear, M. F.; Huber, K. M.; Warren, S. T. The mGluR theory of fragile X mental retardation. *Trends Neurosci.* **2004**, *27*, 370−377.
(38) Paluszkiewicz, S. M.; Martin, B. S.; Huntsman, M. M. Fragile X syndrome: the GABAergic system and circuit dysfunction. *Dev. Neurosci.* **2011**, *33*, 349−364.
(39) Chahrour, M.; Zoghbi, H. Y. The story of Rett syndrome: from clinic to neurobiology. *Neuron* **2007**, *56*, 422−437.
(40) Chao, H. T.; Chen, H.; Samaco, R. C.; Xue, M.; Chahrour, M.; Yoo, J.; Neul, J. L.; Gong, S.; Lu, H. C.; Heintz, N.; Ekker, M.; Rubenstein, J. L.; Noebels, J. L.; Rosenmund, C.; Zoghbi, H. Y. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* **2010**, *468*, 263−269.
(41) Jeste, S. S.; Sahin, M.; Bolton, P.; Ploubidis, J. B.; Humphrey, A. Characterization of autism in young children with tuberous sclerosis complex. *J. Child Neurol.* **2008**, *23*, 520−525.
(42) Han, J. M.; Sahin, M. TSC1/TSC2 signaling in the CNS. *FEBS Lett.* **2011**, *585*, 973−980.
(43) Rubenstein, J. L.; Merzenich, M. M. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes, Brain Behav.* **2003**, *2*, 255−267.
(44) Uzunova, G.; Pallanti, S.; Hollander, E. Excitatory/inhibitory imbalance in autism spectrum disorders: implications for interventions and therapeutics. *World J. Biol. Psychiatry* **2016**, *17*, 174−186.
(45) Yizhar, O.; Fenno, L. E.; Prigge, M.; Schneider, F.; Davidson, T. J.; O'Shea, D. J.; Sohal, V. S.; Goshen, I.; Finkelstein, J.; Paz, J. T.; Stehfest, K.; Fudim, R.; Ramakrishnan, C.; Huguenard, J. R.; Hegemann, P.; Deisseroth, K. Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* **2011**, *477*, 171−178.
(46) Dani, V. S.; Chang, Q.; Maffei, A.; Turrigiano, G. G.; Jaenisch, R.; Nelson, S. B. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 12560−12565.
(47) Pop, A. S.; Levenga, J.; de Esch, C. E.; Buijsen, R. A.; Nieuwenhuizen, I. M.; Li, T.; Isaacs, A.; Gasparini, F.; Oostra, B. A.; Willemsen, R. Rescue of dendritic spine phenotype in Fmr1 KO mice with the mGluR5 antagonist AFQ056/Mavoglurant. *Psychopharmacology* **2014**, *231*, 1227−1235.
(48) Lindsley, C. W.; Emmitte, K. A.; Hopkins, C. R.; Bridges, T. M.; Gregory, K. J.; Niswender, C. M.; Conn, P. J. Practical strategies and concepts in GPCR allosteric modulator discovery: recent advances with metabotropic glutamate receptors. *Chem. Rev.* **2016**, *116*, 6707−6741.
(49) Silverman, J. L.; Tolu, S. S.; Barkan, C. L.; Crawley, J. N. Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. *Neuropsychopharmacology* **2010**, *35*, 976−989.
(50) Silverman, J. L.; Smith, D. G.; Rizzo, S. J.; Karras, M. N.; Turner, S. M.; Tolu, S. S.; Bryce, D. K.; Smith, D. L.; Fonseca, K.; Ring, R. H.; Crawley, J. N. Negative allosteric modulation of the mGluR5 receptor reduces repetitive behaviors and rescues social deficits in mouse models of autism. *Sci. Transl. Med.* **2012**, *4*, 131ra51.
(51) Seese, R. R.; Maske, A. R.; Lynch, G.; Gall, C. M. Long-term memory deficits are associated with elevated synaptic ERK1/2 activation and reversed by mGluR5 antagonism in an animal model of autism. *Neuropsychopharmacology* **2014**, *39*, 1664−1673.
(52) Mehta, M. V.; Gandal, M. J.; Siegel, S. J. mGluR5-antagonist mediated reversal of elevated stereotyped, repetitive behaviors in the VPA model of autism. *PLoS One* **2011**, *6*, e26077.
(53) Rocher, J. P.; Bonnet, B.; Boléa, C.; Lütjens, R.; Le Poul, E.; Poli, S.; Epping-Jordan, M.; Bessis, A. S.; Ludwig, B.; Mutel, V. mGluR5 negative allosteric modulators overview: a medicinal chemistry approach towards a series of novel therapeutic agents. *Curr. Top. Med. Chem.* **2011**, *11*, 680−695.
(54) Emmitte, K. A. Recent advances in the design and development of novel negative allosteric modulators of mGlu5. *ACS Chem. Neurosci.* **2011**, *2*, 411−432.
(55) de Vrij, F. M.; Levenga, J.; van der Linde, H. C.; Koekkoek, S. K.; De Zeeuw, C. I.; Nelson, D. L.; Oostra, B. A.; Willemsen, R. Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 KO mice. *Neurobiol. Dis.* **2008**, *31*, 127−132.
(56) Ceccarelli, S. M.; Jaeschke, G.; Buettelmann, B.; Huwyler, J.; Kolczewski, S.; Peters, J. U.; Prinssen, E.; Porter, R.; Spooren, W.; Vieira, E. Rational design, synthesis, and structure-activity relationship of benzoxazolones: new potent mGlu5 receptor antagonists based on the fenobam structure. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1302−1306.
(57) Vranesic, I.; Ofner, S.; Flor, P. J.; Bilbe, G.; Bouhelal, R.; Enz, A.; Desrayaud, S.; McAllister, K.; Kuhn, R.; Gasparini, F. AFQ056/mavoglurant, a novel clinically effective mGluR5 antagonist: identification, SAR and pharmacological characterization. *Bioorg. Med. Chem.* **2014**, *22*, 5790−5803.
(58) Scharf, S. H.; Jaeschke, G.; Wettstein, J. G.; Lindemann, L. Metabotropic glutamate receptor 5 as drug target for Fragile X syndrome. *Curr. Opin. Pharmacol.* **2015**, *20*, 124−134.

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

(59) Jaeschke, G.; Kolczewski, S.; Spooren, W.; Vieira, E.; Bitter-Stoll, N.; Boissin, P.; Borroni, E.; Büttelmann, B.; Ceccarelli, S.; Clemann, N.; David, B.; Funk, C.; Guba, W.; Harrison, A.; Hartung, T.; Honer, M.; Huwyler, J.; Kuratli, M.; Niederhauser, U.; Pähler, A.; Peters, J. U.; Petersen, A.; Prinssen, E.; Ricci, A.; Rueher, D.; Rueher, M.; Schneider, M.; Spurr, P.; Stoll, T.; Tännler, D.; Wichmann, J.; Porter, R. H.; Wettstein, J. G.; Lindemann, L. Metabotropic glutamate receptor 5 negative allosteric modulators: discovery of 2-chloro-4-[1-(4-fluorophenyl)-2,5-dimethyl-1*H*-imidazol-4-ylethynyl]pyridine (basimglurant, RO4917523), a promising novel medicine for psychiatric diseases. *J. Med. Chem.* **2015**, *58*, 1358−1371.

(60) Munshi, K.; Pawlowski, K.; Gonzalez-Heydrich, J.; Picker, J. D. Review of salient investigational drugs for the treatment of fragile X syndrome. *J. Child Adolesc. Psychopharmacol.* **2017** DOI: 10.1089/cap.2016.0200.

(61) Noetzel, M. J.; Gregory, K. J.; Vinson, P. N.; Manka, J. T.; Stauffer, S. R.; Lindsley, C. W.; Niswender, C. M.; Xiang, Z.; Conn, P. J. A novel metabotropic glutamate receptor 5 positive allosteric modulator acts at a unique site and confers stimulus bias to mGlu5 signaling. *Mol. Pharmacol.* **2013**, *83*, 835−847.

(62) Rook, J. M.; Noetzel, M. J.; Pouliot, W. A.; Bridges, T. M.; Vinson, P. N.; Cho, H. P.; Zhou, Y.; Gogliotti, R. D.; Manka, J. T.; Gregory, K. J.; Stauffer, S. R.; Dudek, F. E.; Xiang, Z.; Niswender, C. M.; Daniels, J. S.; Jones, C. K.; Lindsley, C. W.; Conn, P. J. Unique signaling profiles of positive allosteric modulators of mGlu5 determine differences in in vivo activity. *Biol. Psychiatry* **2013**, *73*, 501−509.

(63) Conde-Ceide, S.; Martinez-Viturro, C. M.; Alcazar, J.; Garcia-Barrantes, P. M.; Lavreysen, H.; Mackie, C.; Vinson, P. N.; Rook, J. M.; Bridges, T. M.; Daniels, S. J.; Megens, A.; Langlois, X.; Drinkenburg, W. H.; Ahnaou, A.; Niswender, C. K.; Jones, C. K.; Macdonald, G. J.; Steckler, T.; Conn, P. J.; Stauffer, S. R.; Bartolomé-Nebreda, J. M.; Lindsley, C. W. Discovery of VU0409551/JNJ-46778212: an mGlu5 positive allosteric modulator clinical candidate targeting schizophrenia. *ACS Med. Chem. Lett.* **2015**, *6*, 716−720.

(64) Chen, Y. W.; Lin, H. C.; Ng, M. C.; Hsiao, Y. H.; Wang, C. C.; Gean, P. W.; Chen, P. S. Activation of mGluR2/3 underlies the effects of N-acetylcystein on amygdala-associated autism-like phenotypes in a valproate-induced rat model of autism. *Front. Behav. Neurosci.* **2014**, *8*, 219.

(65) Monn, J. A.; Valli, M. J.; Massey, S. M.; Hansen, M. M.; Kress, T. J.; Wepsiec, J. P.; Harkness, A. R.; Grutsch, J. L.; Wright, R. A.; Johnson, B. G.; Andis, S. L.; Kingston, A.; Tomlinson, R.; Lewis, R.; Griffey, K. R.; Tizzano, J. P.; Schoepp, D. D. Synthesis, pharmacological characterization, and molecular modeling of heterobicyclic amino acids related to (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740): identification of two new potent, selective, and systemically active agonists for group II metabotropic glutamate receptors. *J. Med. Chem.* **1999**, *42*, 1027−1040.

(66) Ornstein, P. L.; Bleisch, T. J.; Arnold, M. B.; Kennedy, J. H.; Wright, R. A.; Johnson, B. G.; Tizzano, J. P.; Helton, D. R.; Kallman, M. J.; Schoepp, D. D.; Hérin, M. 2-Substituted (2SR)-2-amino-2-((1SR,2SR)-carboxycycloprop-1-yl)glycines as potent and selective antagonists of group II metabotropic glutamate receptors. 2. Effects of aromatic substitution, pharmacological characterization, and bioavailability. *J. Med. Chem.* **1998**, *41*, 358−378.

(67) Chaki, S.; Yoshikawa, R.; Hirota, S.; Shimazaki, T.; Maeda, M.; Kawashima, N.; Yoshimizu, T.; Yasuhara, A.; Sakagami, K.; Okuyama, S.; Nakanishi, S.; Nakazato, A. MGS0039: a potent and selective group II metabotropic glutamate receptor antagonist with antidepressant-like activity. *Neuropharmacology* **2004**, *46*, 457−467.

(68) Johnson, M. P.; Baez, M.; Jagdmann, G. E.; Britton, T. C.; Large, T. H.; Callagaro, D. O.; Tizzano, J. P.; Monn, J. A.; Schoepp, D. D. Discovery of allosteric potentiators for the metabotropic glutamate 2 receptor: synthesis and subtype selectivity of N-(4-(2-methoxyphenoxy)phenyl)-N-(2,2,2-trifluoroethylsulfonyl)pyrid-3-yl-methylamine. *J. Med. Chem.* **2003**, *46*, 3189−3192.

(69) Galici, R.; Jones, C. K.; Hempstapat, K.; Nong, Y.; Echemendia, N. G.; Williams, L. G.; de Paulis, T.; Conn, P. J. Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2 has antipsychotic-and anxiolytic-like effects in mice. *J. Pharmacol. Exp. Ther.* **2006**, *318*, 173−185.

(70) Hiyoshi, T.; Marumo, T.; Hikichi, H.; Tomishima, Y.; Urabe, H.; Tamita, T.; Iida, I.; Yasuhara, A.; Karasawa, J.; Chaki, S. Neurophysiologic and antipsychotic profiles of TASP0433864, a novel positive allosteric modulator of metabotropic glutamate 2 receptor. *J. Pharmacol. Exp. Ther.* **2014**, *351*, 642−653.

(71) Cid, J. M.; Tresadern, G.; Duvey, G.; Lutjens, R.; Finn, T.; Rocher, J. P.; Poli, S.; Vega, J. A.; de Lucas, A. I.; Matesanz, E.; Linares, M. L.; Andrés, J. I.; Alcazar, J.; Alonso, J. M.; Macdonald, G. J.; Oehlrich, D.; Lavreysen, H.; Ahnaou, A.; Drinkenburg, W.; Mackie, C.; Pype, S.; Gallacher, D.; Trabanco, A. A. Discovery of 1-butyl-3-chloro-4-(4-phenyl-1-piperidinyl)-(1*H*)-pyridone (JNJ-40411813): a novel positive allosteric modulator of the metabotropic glutamate 2 receptor. *J. Med. Chem.* **2014**, *57*, 6495−6512.

(72) Cook, D.; Brown, D.; Alexander, R.; March, R.; Morgan, P.; Satterthwaite, G.; Pangalos, M. N. Lessons learned from the fate of AstraZeneca's drug pipeline: a five dimensional framework. *Nat. Rev. Drug Discovery* **2014**, *13*, 419−431.

(73) Campo, B.; Kalinichev, M.; Lambeng, N.; Yacoubi, M. E.; Royer-Urios, I.; Schneider, M.; Legrand, C.; Parron, D.; Girard, F.; Bessif, A.; Poli, S.; Vaugeois, J. M.; Le Poul, E.; Celanire, S. Characterization of an mGluR2/3 negative allosteric modulator in rodent models of depression. *J. Neurogenet.* **2011**, *25*, 152−166.

(74) Homayoun, H.; Stefani, M. R.; Adams, B. W.; Tamagan, G. D.; Moghaddam, B. Functional interaction between NMDA and mGlu5 receptors: effects on working memory, instrumental learning, motor behaviors, and dopamine release. *Neuropsychopharmacology* **2004**, *29*, 1259−1269.

(75) Chung, W.; Choi, S. Y.; Lee, E.; Park, H.; Kang, J.; Park, H.; Choi, Y.; Lee, D.; Park, S. G.; Kim, R.; Cho, Y. S.; Choi, J.; Kim, M. H.; Lee, J. W.; Lee, S.; Rhim, I.; Jung, M. W.; Kim, D.; Bae, Y. C.; Kim, E. Social deficits in IRSp53 mutant mice improved by NMDAR and mGluR5 suppression. *Nat. Neurosci.* **2015**, *18*, 435−443.

(76) Won, H.; Lee, H. R.; Gee, H. Y.; Mah, W.; Kim, J. I.; Lee, J.; Ha, S.; Chung, C.; Jung, E. S.; Cho, Y. S.; Park, S. G.; Lee, J. S.; Lee, K.; Kim, D.; Bae, Y. C.; Kaang, B. K.; Lee, M. G.; Kim, E. Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature* **2012**, *486*, 261−265.

(77) Urbano, M.; Okwara, L.; Manser, P.; Hartmann, K.; Herndon, A.; Deutsch, S. I. A trial of D-cycloserine to treat stereotypies in older adolescents and young adults with autism spectrum disorder. *Clin. Neuropharmacol.* **2014**, *37*, 69−72.

(78) Urbano, M.; Okwara, L.; Manser, P.; Hartmann, K.; Deutsch, S. I. A trial of d-cycloserine to treat the social deficit in older adolescents and young adults with autism spectrum disorders. *J. Neuropsychiatry Clin. Neurosci.* **2015**, *27*, 133−138.

(79) Volkmann, R. A.; Fanger, C. M.; Anderson, D. R.; Sirivolu, V. R.; Paschetto, K.; Gordon, E.; Virginio, C.; Gleyzes, M.; Buisson, B.; Steidl, E.; Mierau, S. B.; Fagiolini, M.; Menniti, F. S. MPX-004 and MPX-007: new pharmacological tools to study the physiology of NMDA receptors containing the GluN2A subunit. *PLoS One* **2016**, *11*, e0148129.

(80) Hussman, J. P. Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. *J. Autism Dev. Disord.* **2001**, *31*, 247−248.

(81) Olsen, R. W.; Sieghart, W. International union of pharmacology. LXX. Subtypes of gamma-aminobutyric acid receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol. Rev.* **2008**, *60*, 243−260.

(82) Coghlan, S.; Horder, J.; Inkster, B.; Mendez, M. A.; Murphy, D. G.; Nutt, D. J. GABA system dysfunction in autism and related disorders: from synapse to symptoms. *Neurosci. Biobehav. Rev.* **2012**, *36*, 2044−2055.

(83) Cellot, G.; Cherubini, E. GABAergic signaling as therapeutic target for autism spectrum disorders. *Front. Pediatr.* **2014**, *2*, 70.

(84) Han, S.; Tai, C.; Jones, C. J.; Scheuer, T.; Catterall, W. A. Enhancement of inhibitory neurotransmission by GABAA receptors

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

having α2,3-subunits ameliorates behavioral deficits in a mouse model of autism. *Neuron* **2014**, *81*, 1282−1289.
(85) Alhambra, C.; Becker, C.; Blake, T.; Chang, A. H.; Damewood, J. R., Jr; Daniels, T.; Dembofsky, B. T.; Gurley, D. A.; Hall, J. E.; Herzog, K. J.; Horchler, C. L.; Ohnmacht, C. J.; Schmiesing, R. J.; Dudley, A.; Ribadeneira, M. D.; Knappenberger, K. S.; Maciag, C.; Stein, M. M.; Chopra, M.; Liu, X. F.; Christian, E. P.; Arriza, J. L.; Chapdelaine, M. J. Development and SAR of functionally selective allosteric modulators of GABAA receptors. *Bioorg. Med. Chem.* **2011**, *19*, 2927−2938.
(86) Brondino, N.; Fusar-Poli, L.; Panisi, C.; Damiani, S.; Barale, F.; Politi, P. Pharmacological modulation of GABA function in autism spectrum disorders: a systematic review of human studies. *J. Autism Dev. Disord.* **2016**, *46*, 825−839.
(87) Berry-Kravis, E. M.; Hessl, D.; Rathmell, B.; Zarevics, P.; Cherubini, M.; Walton-Bowen, K.; Mu, Y.; Nguyen, D. V.; Gonzalez-Heydrich, J.; Wang, P. P.; Carpenter, R. L.; Bear, M. F.; Hagerman, R. J. Effects of STX209 (arbaclofen) on neurobehavioral function in children and adults with fragile X syndrome: a randomized, controlled, phase 2 trial. *Sci. Transl. Med.* **2012**, *4*, 152ra127.
(88) Veenstra-VanderWeele, J.; Cook, E. H.; King, B. H.; Zarevics, P.; Cherubini, M.; Walton-Bowen, K.; Bear, M. F.; Wang, P. P.; Carpenter, R. L. Arbaclofen in children and adolescents with autism spectrum disorder: a randomized, controlled, Phase 2 trial. *Neuropsychopharmacology* **2017**, *42*, 1390−1398.
(89) Brown, K. M.; Roy, K. K.; Hockerman, G. H.; Doerksen, R. J.; Colby, D. A. Activation of the γ-aminobutyric acid type B (GABAB) receptor by agonists and positive allosteric modulators. *J. Med. Chem.* **2015**, *58*, 6336−6347.
(90) Kalinichev, M.; Girard, F.; Haddouk, H.; Rouillier, M.; Riguet, E.; Royer-Urios, I.; Mutel, V.; Lütjens, R.; Poli, S. The drug candidate, ADX71441, is a novel, potent and selective positive allosteric modulator of the GABAB receptor with a potential for treatment of anxiety, pain and spasticity. *Neuropharmacology* **2017**, *114*, 34−47.
(91) Caracci, M. O.; Ávila, M. E.; De Ferrari, G. V. Synaptic Wnt/GSK3β signaling hub in autism. *Neural Plast.* **2016**, *2016*, 9603751.
(92) Mulligan, K. A.; Cheyette, B. N. Neurodevelopmental perspectives on Wnt signaling in psychiatry. *Mol. Neuropsychiatry* **2017**, *2*, 219−246.
(93) Klein, P. S.; Melton, D. A. A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 8455−8459.
(94) Cerpa, W.; Godoy, J. A.; Alfaro, I.; Farías, G. G.; Metcalfe, M. J.; Fuentealba, R.; Bonansco, C.; Inestrosa, N. C. Wnt-7a modulates the synaptic vesicle cycle and synaptic transmission in hippocampal neurons. *J. Biol. Chem.* **2008**, *283*, 5918−5927.
(95) Ahmad-Annuar, A.; Ciani, L.; Simeonidis, I.; Herreros, J.; Fredj, N. B.; Rosso, S. B.; Hall, A.; Brickley, S.; Salinas, P. C. Signaling across the synapse: a role for Wnt and Dishevelled in presynaptic assembly and neurotransmitter release. *J. Cell Biol.* **2006**, *174*, 127−139.
(96) Ciani, L.; Boyle, K. A.; Dickins, E.; Sahores, M.; Anane, D.; Lopes, D. M.; Gibb, A. J.; Salinas, P. C. Wnt7a signaling promotes dendritic spine growth and synaptic strength through Ca^{2+}/Calmodulin-dependent protein kinase II. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 10732−10737.
(97) Latapy, C.; Rioux, V.; Guitton, M. J.; Beaulieu, J. M. Selective deletion of forebrain glycogen synthase kinase 3β reveals a central role in serotonin-sensitive anxiety and social behaviour. *Philos. Trans. R. Soc., B* **2012**, *367*, 2460−2474.
(98) Hernàndez, F.; Borrell, J.; Guaza, C.; Avila, J.; Lucas, J. J. Spatial learning deficit in transgenic mice that conditionally overexpress GSK-3β in the brain but do not form tau filaments. *J. Neurochem.* **2002**, *83*, 1529−1533.
(99) Siegel, M.; Beresford, C. A.; Bunker, M.; Verdi, M.; Vishnevetsky, D.; Karlsson, C.; Teer, O.; Stedman, A.; Smith, K. A. Preliminary investigation of lithium for mood disorder symptoms in children and adolescents with autism spectrum disorder. *J. Child Adolesc. Psychopharmacol.* **2014**, *24*, 399−402.
(100) Wu, X.; Bai, Y.; Tan, T.; Li, H.; Xia, S.; Chang, X.; Zhou, Z.; Zhou, W.; Li, T.; Wang, Y. T.; Dong, Z. Lithium ameliorates autistic-like behaviors induced by neonatal isolation in rats. *Front. Behav. Neurosci.* **2014**, *8*, 234.
(101) Liu, Z.-H.; Huang, T.; Smith, C. B. Lithium reverses increased rates of cerebral protein synthesis in a mouse model of fragile X syndrome. *Neurobiol. Dis.* **2012**, *45*, 1145−1152.
(102) Guo, W.; Murthy, A. C.; Zhang, L.; Johnson, E. B.; Schaller, E. G.; Allan, A. M.; Zhao, X. Inhibition of GSK3β improves hippocampus-dependent learning and rescues neurogenesis in a mouse model of fragile X syndrome. *Hum. Mol. Genet.* **2012**, *21*, 681−691.
(103) Bhat, R.; Xue, Y.; Berg, S.; Hellberg, S.; Ormö, M.; Nilsson, Y.; Radesäter, A. C.; Jerning, E.; Markgren, P. O.; Borgegård, T.; Nylöf, M.; Giménez-Cassina, A.; Hernández, F.; Lucas, J. J.; Díaz-Nido, J.; Avila, J. Structural insights and biological effects of glycogen synthase kinase 3-specific inhibitor AR-A014418. *J. Biol. Chem.* **2003**, *278*, 45937−45945.
(104) Yuskaitis, C. J.; Mines, M. A.; King, M. K.; Sweatt, J. D.; Miller, C. A.; Jope, R. S. Lithium ameliorates altered glycogen synthase kinase-3 and behavior in a mouse model of fragile X syndrome. *Biochem. Pharmacol.* **2010**, *79*, 632−646.
(105) Maqbool, M.; Mobashir, M.; Hoda, N. Pivotal role of glycogen synthase kinase-3: a therapeutic target for Alzheimer's disease. *Eur. J. Med. Chem.* **2016**, *107*, 63−81.
(106) van Spronsen, M.; Hoogenraad, C. C. Synapse pathology in psychiatric and neurologic disease. *Curr. Neurol. Neurosci. Rep.* **2010**, *10*, 207−214.
(107) Berg, J. M.; Geschwind, D. H. Autism genetics: searching for specificity and convergence. *Genome Biol.* **2012**, *13*, 247.
(108) Hutsler, J. J.; Zhang, H. Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Res.* **2010**, *1309*, 83−94.
(109) Geschwind, D. H.; Levitt, P. Autism spectrum disorders: developmental disconnection syndromes. *Curr. Opin. Neurobiol.* **2007**, *17*, 103−111.
(110) Auerbach, B. D.; Osterweil, E. K.; Bear, M. F. Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature* **2011**, *480*, 63−68.
(111) Phillips, M.; Pozzo-Miller, L. Dendritic spine dysgenesis in autism related disorders. *Neurosci. Lett.* **2015**, *601*, 30−40.
(112) Tsai, P.; Sahin, M. Mechanisms of neurocognitive dysfunction and therapeutic considerations in tuberous sclerosis complex. *Curr. Opin. Neurol.* **2011**, *24*, 106−113.
(113) Ishii, R.; Wataya-Kaneda, M.; Canuet, L.; Nonomura, N.; Nakai, Y.; Takeda, M. Everolimus improves behavioral deficits in a patient with autism associated with tuberous sclerosis: a case report. *Neuropsychiatric Electrophysiology* **2015**, *1*, 6.
(114) Huber, K. M.; Klann, E.; Costa-Mattioli, M.; Zukin, R. S. Dysregulation of mammalian target of rapamycin signaling in mouse models of autism. *J. Neurosci.* **2015**, *35*, 13836−13842.
(115) Crino, P. B. Evolving neurobiology of tuberous sclerosis complex. *Acta Neuropathol.* **2013**, *125*, 317−332.
(116) Burket, J. A.; Benson, A. D.; Tang, A. H.; Deutsch, S. I. Rapamycin improves sociability in the BTBR T+Itpr3tf/J mouse model of autism spectrum disorders. *Brain Res. Bull.* **2014**, *100*, 70−75.
(117) Tang, S. J.; Reis, G.; Kang, H.; Gingras, A. C.; Sonenberg, N.; Schuman, E. M. A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 467−472.
(118) Krueger, D. A.; Care, M. M.; Holland, K.; Agricola, K.; Tudor, C.; Mangeshkar, P.; Wilson, K. A.; Byars, A.; Sahmoud, T.; Franz, D. N. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N. Engl. J. Med.* **2010**, *363*, 1801−1811.
(119) Heffron, T. P. Small molecule kinase inhibitors for the treatment of brain cancer. *J. Med. Chem.* **2016**, *59*, 10030−10066.
(120) Fernandez, A. M.; Torres-Alemán, I. The many faces of insulin-like peptide signalling in the brain. *Nat. Rev. Neurosci.* **2012**, *13*, 225−239.

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

(121) Dyer, A. H.; Vahdatpour, C.; Sanfeliu, A.; Tropea, D. The role of insulin-like growth factor 1 (IGF-1) in brain development, maturation and neuroplasticity. *Neuroscience* **2016**, *325*, 89−99.
(122) Cheng, C. M.; Mervis, R. F.; Niu, S. L.; Salem, N., Jr.; Witters, L. A.; Tseng, V.; Reinhardt, R.; Bondy, C. A. Insulin-like growth factor 1 is essential for normal dendritic growth. *J. Neurosci. Res.* **2003**, *73*, 1−9.
(123) Baker, A. M.; Batchelor, D. C.; Thomas, G. B.; Wen, J. Y.; Rafiee, M.; Lin, H.; Guan, J. Central penetration and stability of N-terminal tripeptide of insulin-like growth factor-I, glycine-proline-glutamate in adult rat. *Neuropeptides* **2005**, *39*, 81−87.
(124) Corvin, A. P.; Molinos, I.; Little, G.; Donohoe, G.; Gill, M.; Morris, D. W.; Tropea, D. Insulin-like growth factor 1 (IGF1) and its active peptide (1−3)IGF1 enhance the expression of synaptic markers in neuronal circuits through different cellular mechanisms. *Neurosci. Lett.* **2012**, *520*, 51−56.
(125) Tropea, D.; Giacometti, E.; Wilson, N. R.; Beard, C.; McCurry, C.; Fu, D. D.; Flannery, R.; Jaenisch, R.; Sur, M. Partial reversal of Rett syndrome-like symptoms in MeCP2 mutant mice. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 2029−2034.
(126) Castro, J.; Garcia, R. I.; Kwok, S.; Banerjee, A.; Petravicz, J.; Woodson, J.; Mellios, N.; Tropea, D.; Sur, M. Functional recovery with recombinant human IGF1 treatment in a mouse model of Rett Syndrome. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 9941−9946.
(127) Deacon, R. M.; Glass, L.; Snape, M.; Hurley, M. J.; Altimiras, F. J.; Biekofsky, R. R.; Cogram, P. NNZ-2566, a novel analog of (1−3) IGF-1, as a potential therapeutic agent for fragile X syndrome. *NeuroMol. Med.* **2015**, *17*, 71−82.
(128) Marchetto, M. C.; Belinson, H.; Tian, Y.; Freitas, B. C.; Fu, C.; Vadodaria, K. C.; Beltrao-Braga, P. C.; Trujillo, C. A.; Mendes, A. P. D.; Padmanabhan, K.; Nunez, Y.; Ou, J.; Ghosh, H.; Wright, R.; Brennand, K. J.; Pierce, K.; Eichenfield, L.; Pramparo, T.; Eyler, L. T.; Barnes, C. C.; Courchesne, E.; Geschwind, D. H.; Gage, F. H.; Wynshaw-Boris, A.; Muotri, A. R. Altered proliferation and networks in neural cells derived from idiopathic autistic individuals. *Mol. Psychiatry* **2017**, *22*, 820−835.
(129) Durand, C. M.; Betancur, C.; Boeckers, T. M.; Bockmann, J.; Chaste, P.; Fauchereau, F.; Nygren, G.; Rastam, M.; Gillberg, I. C.; Anckarsäter, H.; Sponheim, E.; Goubran-Botros, H.; Delorme, R.; Chabane, N.; Mouren-Simeoni, M. C.; de Mas, P.; Bieth, E.; Rogé, B.; Héron, D.; Burglen, L.; Gillberg, C.; Leboyer, M.; Bourgeron, T. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat. Genet.* **2007**, *39*, 25−27.
(130) Phelan, M. C.; Rogers, R. C.; Saul, R. A.; Stapleton, G. A.; Sweet, K.; McDermid, H.; Shaw, S. R.; Claytor, J.; Willis, J.; Kelly, D. P. 22q13 deletion syndrome. *Am. J. Med. Genet., Part A* **2001**, *101*, 91−99.
(131) Sarowar, T.; Grabrucker, A. M. Actin-dependent alterations of dendritic spine morphology in shankopathies. *Neural Plast.* **2016**, *2016*, 8051861.
(132) Naisbitt, S.; Kim, E.; Tu, J. C.; Xiao, B.; Sala, C.; Valtschanoff, J.; Weinberg, R. J.; Worley, P. F.; Sheng, M. Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron* **1999**, *23*, 569−582.
(133) Bozdagi, O.; Tavassoli, T.; Buxbaum, J. D. Insulin-like growth favtor-1 rescues synaptic and motor deficits in mouse model of autism and developmental delay. *Mol. Autism* **2013**, *4*, 9.
(134) Shcheglovitov, A.; Shcheglovitova, O.; Yazawa, M.; Portmann, T.; Shu, R.; Sebastiano, V.; Krawisz, A.; Froehlich, W.; Bernstein, J. A.; Hallmayer, J. F.; Dolmetsch, R. E. SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. *Nature* **2013**, *503*, 267−271.
(135) Kolevzon, A.; Bush, L.; Wang, A. T.; Halpern, D.; Frank, Y.; Grodberg, D.; Rapaport, R.; Tavassoli, T.; Chaplin, W.; Soorya, L.; Buxbaum, J. D. A pilot controlled trial of insulin-like growth factor-1 in children with Phelan-McDermid syndrome. *Mol. Autism* **2014**, *5*, 54.
(136) Tu, J. C.; Xiao, B.; Naisbitt, S.; Yuan, J. P.; Petralia, R. S.; Brakeman, P.; Doan, A.; Aakalu, V. K.; Lanahan, A. A.; Sheng, M.; Worley, P. F. Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron* **1999**, *23*, 583−592.
(137) Vicidomini, C.; Ponzoni, L.; Lim, D.; Schmeisser, M. J.; Reim, D.; Morello, N.; Orellana, D.; Tozzi, A.; Durante, V.; Scalmani, P.; Mantegazza, M.; Genazzani, A. A.; Giustetto, M.; Sala, M.; Calabresi, P.; Boeckers, T. M.; Sala, C.; Verpelli, C. Pharmacological enhancement of mGlu5 receptors rescues behavioral deficits in SHANK3 knock-out mice. *Mol. Psychiatry* **2017**, *22*, 784.
(138) Huang, G. H.; Sun, Z. L.; Li, H. J.; Feng, D. F. Rho GTPase-activating proteins: Regulators of Rho GTPase activity in neuronal development and CNS diseases. *Mol. Cell. Neurosci.* **2017**, *80*, 18−31.
(139) Nakagawa, O.; Fujisawa, K.; Ishizaki, T.; Saito, Y.; Nakao, K.; Narumiya, S. ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/ threonine kinase in mice. *FEBS Lett.* **1996**, *392*, 189−193.
(140) Huentelman, M. J.; Stephan, D. A.; Talboom, J.; Corneveaux, J. J.; Reiman, D. M.; Gerber, J. D.; Barnes, C. A.; Alexander, G. E.; Reiman, E. M.; Bimonte-Nelson, H. A. Peripheral delivery of a ROCK inhibitor improves learning and working memory. *Behav. Neurosci.* **2009**, *123*, 218−223.
(141) Swanger, S. A.; Mattheyses, A. L.; Gentry, E. G.; Herskowitz, J. H. ROCK1 and ROCK2 inhibition alters dendritic spine morphology in hippocampal neurons. *Cell Logist.* **2015**, *5*, e1133266.
(142) Alokam, R.; Singhal, S.; Srivathsav, G. S.; Garigipati, S.; Puppala, S.; Sriram, D.; Perumal, Y. Design of dual inhibitors of ROCK-I and NOX2 as potential leads for the treatment of neuroinflammation associated with various neurological diseases including autism spectrum disorder. *Mol. BioSyst.* **2015**, *11*, 607−617.
(143) Feng, Y.; LoGrasso, P. V.; Defert, O.; Li, R. Rho kinase (ROCK) inhibitors and their therapeutic potential. *J. Med. Chem.* **2016**, *59*, 2269−2300.
(144) Hranilovic, D.; Bujas-Petkovic, Z.; Vragovic, R.; Vuk, T.; Hock, K.; Jernej, B. Hyperserotonemia in adults with autistic disorder. *J. Autism Dev. Disord.* **2007**, *37*, 1934−1940.
(145) Coutinho, A. M.; Oliveira, G.; Morgadinho, T.; Fesel, C.; Macedo, T. R.; Bento, C.; Marques, C.; Ataíde, A.; Miguel, T.; Borges, L.; Vicente, A. M. Variants of the serotonin transporter gene (SLC6A4) significantly contribute to hyperserotonemia in autism. *Mol. Psychiatry* **2004**, *9*, 264−271.
(146) Cohen, I. L.; Liu, X.; Schutz, C.; White, B. N.; Jenkins, E. C.; Brown, W. T.; Holden, J. J. A. Association of autism severity with a monoamine oxidase A functional polymorphism. *Clin. Genet.* **2003**, *64*, 190−197.
(147) Veenstra-VanderWeele, J.; Muller, C. L.; Iwamoto, H.; Sauer, J. E.; Owens, W. A.; Shah, C. R.; Cohen, J.; Mannangatti, P.; Jessen, T.; Thompson, B. J.; Ye, R.; Kerr, T. M.; Carneiro, A. M.; Crawley, J. N.; Sanders-Bush, E.; McMahon, D. G.; Ramamoorthy, S.; Daws, L. C.; Sutcliffe, J. S.; Blakely, R. D. Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 5469−5474.
(148) Bortolato, M.; Godar, S. C.; Alzghoul, L.; Zhang, J.; Darling, R. D.; Simpson, K. L.; Bini, V.; Chen, K.; Wellman, C. L.; Lin, R. C.; Shih, J. C. Monoamine oxidase A and A/B knockout mice display autistic-like features. *Int. J. Neuropsychopharmacol.* **2013**, *16*, 869−888.
(149) Muller, C. L.; Anacker, A. M.; Veenstra-VanderWeele, J. The serotonin system in autism spectrum disorder: from biomarker to animal models. *Neuroscience* **2016**, *321*, 24−41.
(150) Williams, K.; Brignell, A.; Randall, M.; Silove, N.; Hazell, P. Selective serotonin reuptake inhibitors (SSRIs) for autism spectrum disorders (ASD). *Cochrane Database Syst. Rev.* **2013**, CD004677.
(151) Hollander, E.; Soorya, L.; Chaplin, W.; Anagnostou, E.; Taylor, B. P.; Ferretti, C. J.; Wasserman, S.; Swanson, E.; Settipani, C. A double-blind placebo-controlled trial of fluoxetine for repetitive behaviors and global severity in adult autism spectrum disorders. *Am. J. Psychiatry* **2012**, *169*, 292−299.
(152) Chugani, D. C.; Chugani, H. T.; Wiznitzer, M.; Parikh, S.; Evans, P. A.; Hansen, R. L.; Nass, R.; Janisse, J. J.; Dixon-Thomas, P.;

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

Behen, M.; Rothermel, R.; Parker, J. S.; Kumar, A.; Muzik, O.; Edwards, D. J.; Hirtz, D.; Jiang, H.; Sivaswamy, L.; Steinhilber, G.; Kennedy, K.; Pawlik, K.; Roeder, R.; Malian, M.; Turner, H.; Hanna, A.; Katusin, J.; Supula, D.; Halverson, S.; Guy, W.; Gjolaj, N.; Mangner, T.; Chakraborty, P.; Wigeluk, A.; Patterson, G.; Mosqueda, A.; Burkett, M.; DeBoard, A.; Cornett, J.; Lee, M.-L.; Lu, X.; Germain, C.; Majkowski, M.; French, B.; Stolinski, A.; Naguib, S.; Wolfe-Christensen, C.; Taylor, S.; Ameli, J.; McQueeney, J. K.; Rubinlicht, M.; Gorka, B.; Zacharski, E.; Blumer, J. L.; Maxwell, K.; Tasi, C.; Toltzis, P.; Kalic, J.; Morus, D.; Paulett, J.; Rosolowski, B.; Banchy, M.; Westlake, K.; Calabro, B.; Tacket, C.; Adkins, I.; Buchheit, K.; Hepper, K.; Speicher, D.; Bergant, S.; Haky, J.; Frohnapfel, M. B.; Schaefer, C.; Browning, A.; Bauer, R.; Embacher, R.; Knapp, J.; Levy, S.; Davies, D.; Lach, D.; Petrich, J.; Kreischer, S.; Blubaugh, P.; Morris, M. A.; Garver, C.; Andersen, M.; Greene, M.; Richards, A.; Rocha, M.; Merryman, R.; Golla, S.; Lorenzen, A.; Sherrod, J. L.; Perryman, P.; Lorduy, K.; Angkustsiri, K.; Plumer, L.B.; Siy Leigh, M. J.; Bacalman, S. C.; Roa, D. L.; Govindappa, K.; Mahajan, G.; Bickel, E.; Brule, N.; Hare, E.; Sarwary, N.; Cargill, D.; Lee, J.; Hom, S.; Steindorf, D.; Pietrykowski, M.; Root, K.; Coffey, S.; Hanzel, E. P.; Santana, A.; Ramirez, C.; Berci, S.; Gabriel, N.; Hirsch, G.; Roberts, E.; Zwilling, A.; Gellatly, R.; Levy, D.; Lee, S.; Rodman, J.; Hanna, A.; Oberfield, N.; Friedland, S.; Lord, C.; Kozloff, R.; Crouse, C.; Millen, S.; Coimbre, A. J.; McCarter, K. L.; Posey, D. Efficacy of low-dose buspirone for restricted and repetitive behavior in young children with autism spectrum disorder: a randomized trial. *J. Pediatr.* **2016**, *170*, 45−53.

(153) Gould, G. G.; Hensler, J. G.; Burke, T. F.; Benno, R. H.; Onaivi, E. S.; Daws, L. C. Density and function of central serotonin (5-HT) transporters, 5-HT_{1A} and 5-HT_{2A} receptors, and effects of their targeting on BTBR T+tf/J mouse social behavior. *J. Neurochem.* **2011**, *116*, 291−303.

(154) Chadman, K. K. Fluoxetine but not risperidone increases sociability in the BTBR mouse model of autism. *Pharmacol., Biochem. Behav.* **2011**, *97*, 586−594.

(155) File, S. E.; Gonzalez, L. E.; Andrews, N. Comparative study of pre- and postsynaptic 5-HT_{1A} receptor modulation of anxiety in two ethological animal tests. *J. Neurosci.* **1996**, *16*, 4810−4815.

(156) Boulougouris, V.; Robbins, T. W. Enhancement of spatial reversal learning by 5-HT_{2C} receptor antagonism is neuroanatomically specific. *J. Neurosci.* **2010**, *30*, 930−938.

(157) Amodeo, D. A.; Jones, J. H.; Sweeney, J. A.; Ragozzino, M. E. Risperidone and the 5-HT_{2A} receptor antagonist M100907 improve probabilistic reversal learning in BTBR T + tf/J mice. *Autism Res.* **2014**, *7*, 555−567.

(158) Amodeo, D. A.; Rivera, E.; Dunn, J. T.; Ragozzino, M. E. M100907 attenuates elevated grooming behavior in the BTBR mouse. *Behav. Brain Res.* **2016**, *313*, 67−70.

(159) Amodeo, D. A.; Rivera, E.; Cook, E. H., Jr.; Sweeney, J. A.; Ragozzino, M. E. 5-HT_{2A} receptor blockade in dorsomedial striatum reduces repetitive behaviors in BTBR mice. *Genes, Brain Behav.* **2017**, *16*, 342−351.

(160) Kvachnina, E.; Liu, G.; Dityatev, A.; Renner, U.; Dumuis, A.; Richter, D. W.; Dityateva, G.; Schachner, M.; Voyno-Yasenetskaya, T. A.; Ponimaskin, E. G. 5-HT_7 receptor is coupled to G alpha subunits of heterotrimeric G12-protein to regulate gene transcription and neuronal morphology. *J. Neurosci.* **2005**, *25*, 7821−7830.

(161) Kobe, F.; Guseva, D.; Jensen, T. P.; Wirth, A.; Renner, U.; Hess, D.; Müller, M.; Medrihan, L.; Zhang, W.; Zhang, M.; Braun, K.; Westerholz, S.; Herzog, A.; Radyushkin, K.; El-Kordi, A.; Ehrenreich, H.; Richter, D. W.; Rusakov, D. A.; Ponimaskin, E. G. 5-HT7R/G12 signaling regulates neuronal morphology and function in an age-dependent manner. *J. Neurosci.* **2012**, *32*, 2915−2930.

(162) Speranza, L.; Chambery, A.; Di Domenico, M.; Crispino, M.; Severino, V.; Volpicelli, F.; Leopoldo, M.; Bellenchi, G. C.; di Porzio, U.; Perrone-Capano, C. The serotonin receptor 7 promotes neurite outgrowth via ERK and Cdk5 signaling pathways. *Neuropharmacology* **2013**, *67*, 155−167.

(163) Speranza, L.; Giuliano, T.; Volpicelli, F.; De Stefano, M. E.; Lombardi, L.; Chambery, A.; Lacivita, E.; Leopoldo, M.; Bellenchi, G. C.; di Porzio, U.; Crispino, M.; Perrone-Capano, C. Activation of 5-HT_7 receptor stimulates neurite elongation through mTOR, Cdc42 and actin filaments dynamics. *Front. Behav. Neurosci.* **2015**, *9*, 62.

(164) Speranza, L.; Labus, J.; Volpicelli, F.; Guseva, D.; Lacivita, E.; Leopoldo, M.; Bellenchi, G. C.; di Porzio, U.; Bijata, M.; Perrone-Capano, C.; Ponimaskin, E. G. Serotonin 5-HT_7 receptor increases the density of dendritic spines and facilitates synaptogenesis in forebrain neurons. *J. Neurochem.* **2017**, *141*, 647−661.

(165) Vizuete, M. L.; Venero, J. L.; Traiffort, E.; Vargas, C.; Machado, A.; Cano, J. Expression of 5-HT_7 receptor mRNA in rat brain during postnatal development. *Neurosci. Lett.* **1997**, *227*, 53−56.

(166) Leo, D.; Adriani, W.; Cavaliere, C.; Cirillo, G.; Marco, E. M.; Romano, E.; di Porzio, U.; Papa, M.; Perrone-Capano, C.; Laviola, G. Methylphenidate to adolescent rats drives enduring changes of accumbal Htr7 expression: implications for impulsive behavior and neuronal morphology. *Genes, Brain Behav.* **2009**, *8*, 356−368.

(167) De Filippis, B.; Nativio, P.; Fabbri, A.; Ricceri, L.; Adriani, W.; Lacivita, E.; Leopoldo, M.; Passarelli, F.; Fuso, A.; Laviola, G. Pharmacological stimulation of the brain serotonin receptor 7 as a novel therapeutic approach for Rett syndrome. *Neuropsychopharmacology* **2014**, *39*, 2506−2518.

(168) De Filippis, B.; Chiodi, V.; Adriani, W.; Lacivita, E.; Mallozzi, C.; Leopoldo, M.; Domenici, M. R.; Fuso, A.; Laviola, G. Long-lasting beneficial effects of central serotonin receptor 7 stimulation in female mice modeling Rett syndrome. *Front. Behav. Neurosci.* **2015**, *9*, 86.

(169) Valenti, D.; de Bari, L.; Vigli, D.; Lacivita, E.; Leopoldo, M.; Laviola, G.; Vacca, R. A.; De Filippis, B. Stimulation of the brain serotonin receptor 7 rescues mitochondrial dysfunction in female mice from two models of Rett syndrome. *Neuropharmacology* **2017**, *121*, 79−88.

(170) Costa, L.; Sardone, L. M.; Lacivita, E.; Leopoldo, M.; Ciranna, L. 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. *Front. Behav. Neurosci.* **2015**, *9*, 65.

(171) Costa, L.; Spatuzza, M.; D'Antoni, S.; Bonaccorso, C. M.; Trovato, C.; Musumeci, S. A.; Leopoldo, M.; Lacivita, E.; Catania, M. V.; Ciranna, L. Activation of 5-HT_7 serotonin receptors reverses metabotropic glutamate receptor-mediated synaptic plasticity in wild-type and Fmr1 knockout mice, a model of Fragile X syndrome. *Biol. Psychiatry* **2012**, *72*, 924−933.

(172) Canal, C. E.; Felsing, D. E.; Liu, Y.; Zhu, W.; Wood, J. T.; Perry, C. K.; Vemula, R.; Booth, R. G. An orally active phenylaminotetralin-chemotype serotonin 5-HT_7 and 5-HT_{1A} receptor partial agonist that corrects motor stereotypy in mouse models. *ACS Chem. Neurosci.* **2015**, *6*, 1259−1270.

(173) Mohler, E. G.; Baker, P. M.; Gannon, K. S.; Jones, S. S.; Shacham, S.; Sweeney, J. A.; Ragozzino, M. E. The effects of PRX-07034, a novel 5-HT_6 antagonist, on cognitive flexibility and working memory in rats. *Psychopharmacology (Berl)* **2012**, *220*, 687−696.

(174) Dayer, A. G.; Jacobshagen, M.; Chaumont-Dubel, S.; Marin, P. 5-HT_6 receptor: a new player controlling the development of neural circuits. *ACS Chem. Neurosci.* **2015**, *6*, 951−960.

(175) Duhr, F.; Déléris, P.; Raynaud, F.; Séveno, M.; Morisset-Lopez, S.; Mannoury la Cour, C.; Millan, M. J.; Bockaert, J.; Marin, P.; Chaumont-Dubel, S. Cdk5 induces constitutive activation of 5-HT_6 receptors to promote neurite growth. *Nat. Chem. Biol.* **2014**, *10*, 590−597.

(176) Caliendo, G.; Santagada, V.; Perissutti, E.; Fiorino, F. Derivatives as 5HT_{1A} receptor ligands−past and present. *Curr. Med. Chem.* **2005**, *12*, 1721−1753.

(177) Middlemiss, D. N.; Tricklebank, M. D. Centrally active 5-HT receptor agonists and antagonists. *Neurosci. Biobehav. Rev.* **1992**, *16*, 75−82.

(178) Leysen, J. E. 5-HT_2 receptors. *Curr. Drug Targets: CNS Neurol. Disord.* **2004**, *3*, 11−26.

(179) Benhamú, B.; Martín-Fontecha, M.; Vázquez-Villa, H.; Pardo, L.; López-Rodríguez, M. L. Serotonin 5-HT_6 receptor antagonists for

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

the treatment of cognitive deficiency in Alzheimer's disease. *J. Med. Chem.* **2014**, *57*, 7160−7181.
(180) Leopoldo, M.; Lacivita, E.; Berardi, F.; Perrone, R.; Hedlund, P. B. Serotonin 5-HT_7 receptor agents: Structure-activity relationships and potential therapeutic applications in central nervous system disorders. *Pharmacol. Ther.* **2011**, *129*, 120−148.
(181) Karvat, G.; Kimchi, T. Acetylcholine elevation relieves cognitive rigidity and social deficiency in a mouse model of autism. *Neuropsychopharmacology* **2014**, *39*, 831−840.
(182) Deutsch, S. I.; Burket, J. A.; Urbano, M. R.; Benson, A. D. The α7 nicotinic acetylcholine receptor: a mediator of pathogenesis and therapeutic target in autism spectrum disorders and Down syndrome. *Biochem. Pharmacol.* **2015**, *97*, 363−377.
(183) Deutsch, S. I.; Urbano, M. R.; Burket, J. A.; Herndon, A. L.; Winebarger, E. E. Pharmacotherapeutic implications of the association between genomic instability at chromosome 15q13.3 and autism spectrum disorders. *Clin. Neuropharmacol.* **2011**, *34*, 203−205.
(184) Jones, S.; Sudweeks, S.; Yakel, J. L. Nicotinic receptors in the brain: correlating physiology with function. *Trends Neurosci.* **1999**, *22*, 555−561.
(185) Beinat, C.; Banister, S. D.; Herrera, M.; Kassiou, M. The recent development of α7 nicotinic acetylcholine receptor (nAChR) ligands as therapeutic candidates for the treatment of central nervous system (CNS) diseases. *Curr. Pharm. Des.* **2016**, *22*, 2134−2151.
(186) Jones, C. K.; Byun, N.; Bubser, M. Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. *Neuropsychopharmacology* **2012**, *37*, 16−42.
(187) Hurst, R. S.; Hajós, M.; Raggenbass, M.; Wall, T. M.; Higdon, N. R.; Lawson, J. A.; Rutherford-Root, K. L.; Berkenpas, M. B.; Hoffmann, W. E.; Piotrowski, D. W.; Groppi, V. E.; Allaman, G.; Ogier, R.; Bertrand, S.; Bertrand, D.; Arneric, S. P. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. *J. Neurosci.* **2005**, *25*, 4396−4405.
(188) Faghih, R.; Gopalakrishnan, S. M.; Gronlien, J. H.; Malysz, J.; Briggs, C. A.; Wetterstrand, C.; Ween, H.; Curtis, M. P.; Sarris, K. A.; Gfesser, G. A.; El-Kouhen, R.; Robb, H. M.; Radek, R. J.; Marsh, K. C.; Bunnelle, W. H.; Gopalakrishnan, M. Discovery of 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1H-pyrrol-1-yl)-benzenesulfonamide (A-867744) as a novel positive allosteric modulator of the alpha7 nicotinic acetylcholine receptor. *J. Med. Chem.* **2009**, *52*, 3377−3384.
(189) Carter, C. S.; Williams, J. R.; Witt, D. M.; Insel, T. R. Oxytocin and social bonding. *Ann. N. Y. Acad. Sci.* **1992**, *652*, 204−211.
(190) Insel, T. R. A neurobiological basis of social attachment. *Am. J. Psychiatry* **1997**, *154*, 726−735.
(191) Modahl, C.; Green, L.; Fein, D.; Morris, M.; Waterhouse, L.; Feinstein, C.; Levin, H. Plasma oxytocin levels in autistic children. *Biol. Psychiatry* **1998**, *43*, 270−277.
(192) Green, L.; Fein, D.; Modahl, C.; Feinstein, C.; Waterhouse, L.; Morris, M. Oxytocin and autistic disorder: alterations in peptide forms. *Biol. Psychiatry* **2001**, *50*, 609−613.
(193) LoParo, D.; Waldman, I. D. The oxytocin receptor gene (OXTR) is associated with autism spectrum disorder: a meta-analysis. *Mol. Psychiatry* **2015**, *20*, 640−646.
(194) Jin, D.; Liu, H. X.; Hirai, H.; Torashima, T.; Nagai, T.; Lopatina, O.; Shnayder, N. A.; Yamada, K.; Noda, M.; Seike, T.; Fujita, K.; Takasawa, S.; Yokoyama, S.; Koizumi, K.; Shiraishi, Y.; Tanaka, S.; Hashii, M.; Yoshihara, T.; Higashida, K.; Islam, M. S.; Yamada, N.; Hayashi, K.; Noguchi, N.; Kato, I.; Okamoto, H.; Matsushima, A.; Salmina, A.; Munesue, T.; Shimizu, N.; Mochida, S.; Asano, M.; Higashida, H. CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature* **2007**, *446*, 41−45.
(195) Preti, A.; Melis, M.; Siddi, S.; Vellante, M.; Doneddu, G.; Fadda, R. Oxytocin and autism: a systematic review of randomized controlled trials. *J. Child Adolesc. Psychopharmacol.* **2014**, *24*, 54−68.
(196) Leng, G.; Ludwig, M. Intranasal oxytocin: myths and delusions. *Biol. Psychiatry* **2016**, *79*, 243−250.
(197) Wiśniewski, K.; Alagarsamy, S.; Galyean, R.; Tariga, H.; Thompson, D.; Ly, B.; Wiśniewska, H.; Qi, S.; Croston, G.; Laporte, R.; Rivière, P. J.; Schteingart, C. D. New, potent, and selective peptidic oxytocin receptor agonists. *J. Med. Chem.* **2014**, *57*, 5306−5317.
(198) Ashworth, D. M.; Batt, A. R.; Baxter, A. J.; Broqua, P.; Haigh, R. M.; Hudson, P.; Heeney, C. M.; Laporte, R.; Penson, A. M.; Pitt, G. R.; Robson, P. A.; Rooker, D. P.; Tartar, A. L.; Yea, C. M.; Roe, M. B. Nonpeptide oxytocin agonists. *Drugs Future* **2006**, *31*, 345−354.
(199) Ring, R. H.; Schechter, L. E.; Leonard, S. K.; Dwyer, J. M.; Platt, B. J.; Graf, R.; Grauer, S.; Pulicicchio, C.; Resnick, L.; Rahman, Z.; Sukoff Rizzo, S. J.; Luo, B.; Beyer, C. E.; Logue, S. F.; Marquis, K. L.; Hughes, Z. A.; Rosenzweig-Lipson, S. Receptor and behavioral pharmacology of WAY-267464, a non-peptide oxytocin receptor agonist. *Neuropharmacology* **2010**, *58*, 69−77.
(200) Pitt, G. R.; Batt, A. R.; Haigh, R. M.; Penson, A. M.; Robson, P. A.; Rooker, D. P.; Tartar, A. L.; Trim, J. E.; Yea, C. M.; Roe, M. B. Non-peptide oxytocin agonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4585−4589.
(201) Frantz, M. C.; Rodrigo, J.; Boudier, L.; Durroux, T.; Mouillac, B.; Hibert, M. Subtlety of the structure-affinity and structure-efficacy relationships around a nonpeptide oxytocin receptor agonist. *J. Med. Chem.* **2010**, *53*, 1546−1562.
(202) Bissantz, C.; Grundschober, C.; Nettekoven, M.; Plancher, J. M.; Vifian, W. Oxytocin Receptor Agonists for the Treatment of CNS Diseases. WIPO Patent WO2014111356, 2014.
(203) Izdebska, K.; Ciosek, J. Galanin influences on vasopressin and oxytocin release: in vitro studies. *Neuropeptides* **2010**, *44*, 341−348.
(204) Riebold, M.; Mankuta, D.; Lerer, E.; Israel, S.; Zhong, S.; Nemanov, L.; Monakhov, M. V.; Levi, S.; Yirmiya, N.; Yaari, M.; Malavasi, F.; Ebstein, R. P. All-trans retinoic acid upregulates reduced CD38 transcription in lymphoblastoid cell lines from autism spectrum disorder. *Mol. Med.* **2011**, *17*, 799−806.
(205) Sabatier, N. Alpha-melanocyte-stimulating hormone and oxytocin: a peptide signalling cascade in the hypothalamus. *J. Neuroendocrinol.* **2006**, *18*, 703−710.
(206) Peñagarikano, O.; Lázaro, M. T.; Lu, X. H.; Gordon, A.; Dong, H.; Lam, H. A.; Peles, E.; Maidment, N. T.; Murphy, N. P.; Yang, X. W.; Golshani, P.; Geschwind, D. H. Exogenous and evoked oxytocin restores social behavior in the Cntnap2 mouse model of autism. *Sci. Transl. Med.* **2015**, *7*, 271ra8.
(207) Jørgensen, H.; Riis, M.; Knigge, U.; Kjaer, A.; Warberg, J. Serotonin receptors involved in vasopressin and oxytocin secretion. *J. Neuroendocrinol.* **2003**, *15*, 242−249.
(208) Bagdy, G.; Kalogeras, K. T. Stimulation of 5-HT_{1A} and 5-HT_2/5-HT_{1C} receptors induce oxytocin release in the male rat. *Brain Res.* **1993**, *611*, 330−332.
(209) Uvnäs-Moberg, K.; Hillegaart, V.; Alster, P.; Ahlenius, S. Effects of 5-HT agonists, selective for different receptor subtypes, on oxytocin, CCK, gastrin and somatostatin plasma levels in the rat. *Neuropharmacology* **1996**, *35*, 1635−1640.
(210) Lawson, S. K.; Gray, A. C.; Woehrle, N. S. Effects of oxytocin on serotonin 1B agonist-induced autism-like behavior in mice. *Behav. Brain Res.* **2016**, *314*, 52−64.
(211) Matsumoto, H.; Nagasaka, T.; Hattori, A.; Rogi, T.; Tsuruoka, N.; Mizutani, S.; Tsujimoto, M. Expression of placental leucine aminopeptidase/oxytocinase in neuronal cells and its action on neuronal peptides. *Eur. J. Biochem.* **2001**, *268*, 3259−3266.
(212) Albiston, A. L.; Diwakarla, S.; Fernando, R. N.; Mountford, S. J.; Yeatman, H. R.; Morgan, B.; Pham, V.; Holien, J. K.; Parker, M. W.; Thompson, P. E.; Chai, S. Y. Identification and development of specific inhibitors for insulin-regulated aminopeptidase as a new class of cognitive enhancers. *Br. J. Pharmacol.* **2011**, *164*, 37−47.
(213) Insel, T. R. The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. *Neuron* **2010**, *65*, 768−779.
(214) Ebner, K.; Wotjak, C. T.; Landgraf, R.; Engelmann, M. Forced swimming triggers vasopressin release within the amygdala to modulate stress-coping strategies in rats. *Eur. J. Neurosci.* **2002**, *15*, 384−388.

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

(215) Zink, C. F.; Stein, J. L.; Kempf, L.; Hakimi, S.; Meyer-Lindenberg, A. Vasopressin modulates medial prefrontal cortex−amygdala circuitry during emotion processing in humans. *J. Neurosci.* **2010**, *30*, 7017−7022.
(216) Loup, F.; Tribollet, E.; Dubois-Dauphin, M.; Dreifuss, J. J. Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. *Brain Res.* **1991**, *555*, 220−232.
(217) Ratni, H.; Rogers-Evans, M.; Bissantz, C.; Grundschober, C.; Moreau, J. L.; Schuler, F.; Fischer, H.; Alvarez Sanchez, R.; Schnider, P. Discovery of highly selective brain-penetrant vasopressin 1a antagonists for the potential treatment of autism via a chemogenomic and scaffold hopping approach. *J. Med. Chem.* **2015**, *58*, 2275−2289.
(218) Umbricht, D.; Del Valle Rubido, M.; Hollander, E.; McCracken, J. T.; Shic, F.; Scahill, L.; Noeldeke, J.; Boak, L.; Khwaja, O.; Squassante, L.; Grundschober, C.; Kletzl, H.; Fontoura, P. A single dose, randomized, controlled proof-of-mechanism study of a novel vasopressin 1a receptor antagonist (RG7713) in high-functioning adults with autism spectrum disorder. *Neuropsychopharmacology* **2017**, *42*, 1914−1923.
(219) O'Dushlaine, C.; The Network and Pathway Analysis Subgroup of the Psychiatric Genomics Consortium. Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways. *Nat. Neurosci.* **2015**, *18*, 199−209.
(220) Merrill, J. E. Tumor necrosis factor alpha, interleukin 1 and related cytokines in brain development: normal and pathological. *Dev. Neurosci.* **2004**, *14*, 1−10.
(221) Miller, L. C.; Isa, S.; LoPreste, G.; Schaller, J. G.; Dinarello, C. A. Neonatal interleukin- 1 beta, interleukin-6, and tumor necrosis factor: cord blood levels and cellular production. *J. Pediatr.* **1990**, *117*, 961−965.
(222) Yoon, B. H.; Romero, R.; Kim, C. J.; Jun, J. K.; Gomez, R.; Choi, J. H.; Syu, H. C. Amniotic fluid interleukin-6: a sensitive test for antenatal diagnosis of acute inflammatory lesions of preterm placenta and prediction of perinatal morbidity. *Am. J. Obstet. Gynecol.* **1995**, *172*, 960−970.
(223) Abbott, N. J.; Patabendige, A. A.; Dolman, D. E.; Yusof, S. R.; Begley, D. J. Structure and function of the blood-brain barrier. *Neurobiol. Dis.* **2010**, *37*, 13−25.
(224) Malik, M.; Sheikh, A. M.; Wen, G.; Spivack, W.; Brown, W. T.; Li, X. Expression of inflammatory cytokines, Bcl2 and cathepsin D are altered in lymphoblasts of autistic subjects. *Immunobiology* **2011**, *216*, 80−85.
(225) Depino, A. M. Peripheral and central inflammation in autism spectrum disorders. *Mol. Cell. Neurosci.* **2013**, *53*, 69−76.
(226) Auld, D. S.; Robitaille, R. Glial cells and neurotransmission: an inclusive view of synaptic function. *Neuron* **2003**, *40*, 389−400.
(227) Steinmetz, C. C.; Buard, I.; Claudepierre, T.; Nägler, K.; Pfrieger, F. W. Regional variations in the glial influence on synapse development in the mouse CNS. *J. Physiol.* **2006**, *577*, 249−261.
(228) Verkhratsky, A. Physiology of neuronal−glial networking. *Neurochem. Int.* **2010**, *57*, 332−343.
(229) Minghetti, L.; Carnevale, D.; De Simone, R. Microglia−neuron interaction in inflammatory and degenerative diseases: role of cholinergic and noradrenergic systems. *CNS Neurol. Disord.: Drug Targets* **2007**, *6*, 388−397.
(230) Byrnes, K. R.; Stoica, B.; Loane, D. J.; Riccio, A.; Davis, M. I.; Faden, A. I. Metabotropic glutamate receptor 5 activation inhibits microglial associated inflammation and neurotoxicity. *Glia* **2009**, *57*, 550−560.
(231) Loane, D. J.; Stoica, B. A.; Pajoohesh-Ganji, A.; Byrnes, K. R.; Faden, A. I. Activation of metabotropic glutamate receptor 5 modulates microglial reactivity and neurotoxicity by inhibiting NADPH oxidase. *J. Biol. Chem.* **2009**, *284*, 15629−15639.
(232) Drouin-Ouellet, J.; Brownell, A. L.; Saint-Pierre, M.; Fasano, C.; Emond, V.; Trudeau, L. E.; Levesque, D.; Cicchetti, F. Neuroinflammation is associated with changes in glial mGluR5 expression and the development of neonatal excitotoxic lesions. *Glia* **2011**, *59*, 188−199.
(233) Bergeron, J. D.; Deslauriers, J.; Grignon, S.; Fortier, L. C.; Lepage, M.; Stroh, T.; Poyart, C.; Sébire, G. White matter injury and autistic-like behavior predominantly affecting male rat offspring exposed to group B streptococcal maternal inflammation. *Dev. Neurosci.* **2013**, *35*, 504−515.
(234) Le Belle, J. E.; Sperry, J.; Ngo, A.; Ghochani, Y.; Laks, D. R.; López-Aranda, M.; Silva, A. J.; Kornblum, H. I. Maternal inflammation contributes to brain overgrowth and autism-associated behaviors through altered redox signaling in stem and progenitor cells. *Stem Cell Rep.* **2014**, *3*, 725−734.
(235) Zhou, Y.; Guo, M.; Wang, X.; Li, J.; Wang, Y.; Ye, L.; Dai, M.; Zhou, L.; Persidsky, Y.; Ho, W. TLR3 activation efficiency by high or low molecular mass poly I:C. *Innate Immun.* **2013**, *19*, 184−192.
(236) Holm, T. H.; Draeby, D.; Owens, T. Microglia are required for astroglial Toll-like receptor 4 response and for optimal TLR2 and TLR3 response. *Glia* **2012**, *60*, 630−638.
(237) Nadeem, A.; Ahmad, S. F.; Bakheet, S. A.; Al-Harbi, N. O.; Al-Ayadhi, L. Y.; Attia, S. M.; Zoheir, K. M. Toll-like receptor 4 signaling is associated with upregulated NADPH oxidase expression in peripheral T cells of children with autism. *Brain, Behav., Immun.* **2017**, *61*, 146−154.
(238) Naik, U. S.; Gangadharan, C.; Abbagani, K.; Nagalla, B.; Dasari, N.; Manna, S. K. A study of nuclear transcription factor-kappa B in childhood autism. *PLoS One* **2011**, *6*, e19488.
(239) Li, J.; Csakai, A.; Jin, J.; Zhang, F.; Yin, H. Therapeutic developments targeting Toll-like receptor-4-mediated neuroinflammation. *ChemMedChem* **2016**, *11*, 154−165.
(240) Gárate, I.; García-Bueno, B.; Madrigal, J. L.; Caso, J. R.; Alou, L.; Gómez-Lus, M. L.; Leza, J. C. Toll-like 4 receptor inhibitor TAK-242 decreases neuroinflammation in rat brain frontal cortex after stress. *J. Neuroinflammation* **2014**, *11*, 8.
(241) Hutchinson, M. R.; Loram, L. C.; Zhang, Y.; Shridhar, M.; Rezvani, N.; Berkelhammer, D.; Phipps, S.; Foster, P. S.; Landgraf, K.; Falke, J. J.; Rice, K. C.; Maier, S. F.; Yin, H.; Watkins, L. R. Evidence that tricyclic small molecules may possess toll-like receptor and myeloid differentiation protein 2 activity. *Neuroscience* **2010**, *168*, 551−563.
(242) Jaiswal, P.; Mohanakumar, K. P.; Rajamma, U. Serotonin mediated immunoregulation and neural functions: complicity in the aetiology of autism spectrum disorders. *Neurosci. Biobehav. Rev.* **2015**, *55*, 413−431.
(243) Cloez-Tayarani, I.; Petit-Bertron, A. F.; Venters, H. D.; Cavaillon, J. M. Differential effect of serotonin on cytokine production in lipopolysaccharide-stimulated human peripheral blood mononuclear cells: involvement of 5-hydroxytryptamine2A receptors. *Int. Immunol.* **2003**, *15*, 233−240.
(244) Baganz, N. L.; Blakely, R. D. A dialogue between the immune system and brain, spoken in the language of serotonin. *ACS Chem. Neurosci.* **2013**, *4*, 48−63.
(245) Zhu, C. B.; Carneiro, A. M.; Dostmann, W. R.; Hewlett, W. A.; Blakely, R. D. p38 MAPK activation elevates serotonin transport activity via a trafficking-independent, protein phosphatase 2A-dependent process. *J. Biol. Chem.* **2005**, *280*, 15649−15658.
(246) Angelidou, A.; Francis, K.; Vasiadi, M.; Alysandratos, K. D.; Zhang, B.; Theoharides, A.; Lykouras, L.; Sideri, K.; Kalogeromitros, D.; Theoharides, T. C. Neurotensin is increased in serum of young children with autistic disorder. *J. Neuroinflammation* **2010**, *7*, 48.
(247) Patel, A. B.; Tsilioni, I.; Leeman, S. E.; Theoharides, T. C. Neurotensin stimulates sortilin and mTOR in human microglia inhibitable by methoxyluteolin, a potential therapeutic target for autism. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, E7049−E7058.
(248) Nykjaer, A.; Willnow, T. E. Sortilin: a receptor to regulate neuronal viability and function. *Trends Neurosci.* **2012**, *35*, 261−270.
(249) Hermans-Borgmeyer, I.; Hermey, G.; Nykjaer, A.; Schaller, C. Expression of the 100-kDa neurotensin receptor sortilin during mouse embryonal development. *Mol. Brain Res.* **1999**, *65*, 216−219.
(250) Theoharides, T. C.; Valent, P.; Akin, C. Mast cells, mastocytosis, and related disorders. *N. Engl. J. Med.* **2015**, *373*, 163−172.

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX

(251) Wilson, C. M.; Naves, T.; Saada, S.; Pinet, S.; Vincent, F.; Lalloué, F.; Jauberteau, M. O. The implications of sortilin/vps10p domain receptors in neurological and human diseases. *CNS Neurol. Disord.: Drug Targets* **2014**, *13*, 1354−1365.
(252) Schrøder, T. J.; Christensen, S.; Lindberg, S.; Langgård, M.; David, L.; Maltas, P. J.; Eskildsen, J.; Jacobsen, J.; Tagmose, L.; Simonsen, K. B.; Biilmann Rønn, L. C.; de Jong, I. E.; Malik, I. J.; Karlsson, J. J.; Bundgaard, C.; Egebjerg, J.; Stavenhagen, J. B.; Strandbygård, D.; Thirup, S.; Andersen, J. L.; Uppalanchi, S.; Pervaram, S.; Kasturi, S. P.; Eradi, P.; Sakumudi, D. R.; Watson, S. P. The identification of AF38469: an orally bioavailable inhibitor of the VPS10P family sorting receptor Sortilin. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 177−180.
(253) Andersen, J. L.; Lindberg, S.; Langgård, M.; Maltas, P. J.; Rønn, L. C. B.; Bundgaard, C.; Strandbygaard, D.; Thirup, S.; Watson, S. P. The identification of novel acid isostere based inhibitors of the VPS10P family sorting receptor sortilin. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2629−2633.
(254) Peng, W.; Yan, J.; Wan, Y.; Wang, B. B.; Tao, J.; Yang, G.; Pan, H. J. W.; Wang, J. Matrix metalloproteinases: a review of their structure and role in systemic sclerosis. *J. Clin. Immunol.* **2012**, *32*, 1409−1414.
(255) Abdallah, M. W.; Michel, T. M. Matrix metalloproteinases in autism spectrum disorders. *J. Mol. Psychiatry* **2013**, *1*, 16.
(256) Bilousova, T. V.; Dansie, L.; Ngo, M.; Aye, J.; Charles, J. R.; Ethell, D. W.; Ethell, I. M. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *J. Med. Genet.* **2008**, *46*, 94−102.
(257) Dziembowska, M.; Pretto, D. I.; Janusz, A.; Kaczmarek, L.; Leigh, M. J.; Gabriel, N.; Durbin-Johnson, B.; Hagerman, R. J.; Tassone, F. High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. *Am. J. Med. Genet., Part A* **2013**, *161*, 1897−1903.
(258) Geschwind, D. H. Autism: many genes, common pathways? *Cell* **2008**, *135*, 391−395.
(259) Lim, C. S.; Yang, J. E.; Lee, Y. K.; Lee, K.; Lee, J. A.; Kaang, B. K. Understanding the molecular basis of autism in a dish using hiPSCs-derived neurons from ASD patients. *Mol. Brain* **2015**, *8*, 57.
(260) Nargund, R. P.; Strack, A. M.; Fong, T. M. Melanocortin-4 receptor (MC4R) agonists for the treatment of obesity. *J. Med. Chem.* **2006**, *49*, 4035−4043.
(261) King, J. R.; Kabbani, N. Alpha 7 nicotinic receptor coupling to heterotrimeric G proteins modulates RhoA activation, cytoskeletal motility, and structural growth. *J. Neurochem.* **2016**, *138*, 532−545.
(262) Loth, E.; Murphy, D. G.; Spooren, W. Defining precision medicine approaches to autism spectrum disorders: concepts and challenges. *Front. Psychiatry* **2016**, *7*, 188.

DOI: 10.1021/acs.jmedchem.7b00965
J. Med. Chem. XXXX, XXX, XXX−XXX