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A novel potential therapeutic avenue for autism: Design, synthesis and pharmacophore generation of SSRIs with dual action

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Abstract

Autism symptoms are currently modulated by Selective Serotonin Reuptake Inhibitors (SSRIs). SSRIs slow onset of action limits their efficiency. The established synergistic activity of SSRIs and 5HT_{1B/1D} autoreceptors antagonists motivated us to incorporate SSRIs and 5HT_{1B/1D} antagonists in one ‘hybrid’ molecule. A library of virtual ‘hybrid’ molecules was designed using the tethering technique. A pharmacophore model was generated derived from 16 structurally diverse SSRIs ($K_i = 0.013$ –5000 nM) and used as 3D query. Compounds with fit values (> 2) were chosen for synthesis and subsequent in vitro biological evaluation. Our pharmacophore model is a promising milestone to a class of SSRIs with dual action.

Keywords

Autism; Selective Serotonin Reuptake Inhibitors (SSRIs); Pharmacophore

Autism is a poorly understood neurodevelopmental disorder that was first described by Kanner in 1943.¹ Autism prevalence has significantly increased worldwide from 1 case per 10,000 children in the early 1990's to reach 1 case per 110 children in 2010 according to the Centers for Disease Control and Prevention.² Autism manifests itself by three main domains; social skills deficit, communication impairments, and repetitive behaviors. Selective Serotonin Reuptake Inhibitors (SSRIs) are currently the drug class of choice for treating autism symptoms including repetitive behaviors.³ SSRIs are originally synthetic

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Supplementary data

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antidepressant agents with established efficacy for the management of anxiety, obsessive compulsive disorder (OCD) and recently employed for reducing or modulating repetitive behavior symptoms in autistic children.⁴ As a major drawback, it takes 3–6 weeks for SSRIs to become therapeutically efficient. The delay in efficiency varies from one individual to another and significantly decreases compliance, and increases anxiety level about the effectiveness of the undergoing therapy.⁵ An explanation for the delay in efficiency is attributed to the mechanism by which SSRIs operate. SSRIs permeate across the blood–brain barrier and inhibit the neurotransmitter serotonin (5-HT) binding to its transporter, which blocks 5-HT reuptake and increase its concentration in the synaptic cleft (Fig. 1, Panel A). The elevated concentration of 5-HT in the synaptic cleft stimulates postsynaptic 5-HT receptors to initiate a cascade of pharmacological effects that relieve autism symptoms including repetitive behaviors and restricted interests.⁶ However, the elevated concentrations of 5-HT in the synaptic cleft also stimulates inhibitory presynaptic 5-HT autoreceptors (e.g., 5-HT_{1B/1D}). Inhibitory receptors, by definition, regulate the release of 5-HT in an inhibitory manner and negatively affect the mode of action of SSRIs and delay the therapeutic effect of SSRIs for several weeks after their administration.^{4,5} Acute blockade of the inhibitory autoreceptors proved to eliminate their negative feedback effect on 5-HT release and synergistically increased the efficiency of SSRIs (Fig. 1, Panel B). Accordingly, co-administration of SSRIs with 5-HT_{1B/1D} receptor antagonists is advantageous over SSRIs alone with respect to the magnitude of extracellular brain 5-HT levels produced.^{6,7} The established synergistic activity of SSRIs and autoreceptors antagonists motivated us to incorporate 5-HT reuptake inhibitors and antagonists of the 5-HT_{1B/1D} autoreceptors in one molecule to develop ‘hybrid’ SSRIs with dual action as anti-autism candidates. We hypothesized that “hybrid” anti-autism drug candidates will exhibit fast and efficient inhibition of 5-HT reuptake, which will result in a rapid and consistent increase in 5-HT concentration in the synaptic cleft and a corresponding quick control over autism symptoms. Development of hybrid anti-autism drugs will eliminate the need for co-administration of SSRIs and 5-HT autoreceptor antagonists and the associated high risk of dual pharmacokinetics/dosage/side effect and poor overall patient compliance. In our journey to discover hybrid anti-autism candidates, we report herein the design, pharmacophore generation and the chemical synthesis of ‘hybrid’ SSRIs with dual action. To the best of our knowledge, this is an unprecedented therapeutic avenue for modulating autism symptoms.

Our approach for developing ‘hybrid’ small molecules with dual action is based on the tethering technique (Fragment based drug discovery).⁸ Ideally, we identify the molecular scaffold(s) responsible for the desired pharmacological action(s) and link the two scaffolds together via an appropriate covalent linker.

Molecular scaffolds of 5-HT_{1B/1D} Antagonists

GR127935 was the first reported potent 5-HT_{1B/1D} antagonist (Fig. 2), followed by several other potent compounds.⁹ Few ligands were successfully synthesized with selectivity towards either the 5-HT_{1B} or 5-HT_{1D} receptor subtype. For example, *O*-tolylpiperazide and the lead compound reported by Huang et al. are specific antagonists for the 5-HT_{1B} receptor (Fig. 2).¹⁰ BRL-15,572 was developed as an antagonist for 5-HT_{1D} receptor (Fig. 2).¹¹ By critically analyzing the structure of these 5-HT_{1B/1D} antagonists, one can recognize that the

N-(4-methoxyphenyl)amide group with and without the 4-methylpiperazinyl moiety (red group, Fig. 2) to be a common molecular scaffolds in GR127935, Huang's lead compound, and others (not shown). Substitution of the carboxylic side of the amide group tolerates a wide variety of bulky, aromatic, and nonaromatic substituents without affecting the binding affinity of these molecules to 5HT_{1B/1D} receptors.¹² Earlier studies clearly showed that substitution with functionalized arylpiperazine groups into a series of nonselective 5-HT₁ ligands resulted in their selective binding to 5-HT_{1B/1D} receptors versus other 5-HT₁ subtypes.¹² These results together with earlier findings suggest that 5-HT_{1B/1D} receptors possess a deep binding pocket in the binding domain that recognizes the bulky substitution with the arylpiperazine group on the serotonin ring. Specifically, this region of bulk tolerance seems to differentiate between different 5-HT₁ receptor subtypes and allows preferential binding to 5-HT_{1B/1D} receptor subtypes (Fig. 2).^{6,12} From the above, we can conclude that both *N*-(4-methoxyphenyl)amide group with/without 4-methylpiperazine and functionalized arylpiperazine moieties are promising molecular scaffolds for development of 5-HT_{1B/1D} selective antagonists (red group in Figs. 2 and 4). We have utilized these moieties to function as selective antagonists for 5-HT_{1B/1D} receptors in our 'hybrid' molecules.

Molecular scaffolds of 5-HT reuptake inhibitors

Compared to 5-HT_{1B/1D} antagonists, examining the chemical structure of SSRIs did not reveal an obvious structural feature that may be responsible for their activity. The wide structural diversity of SSRIs (Fig. 3) suggests that their binding interaction to the Serotonin Reuptake Transporter (SERT) is quite flexible.¹³ By analyzing the structure of sertraline and paroxetine (Fig. 3), one easily recognizes that sertraline has a halogenated phenyltetralin moiety with 2 chiral centers, whereas the main nucleus of paroxetine is a halogenated phenylpiperidine with one chiral center. One approach to obtain a more rigid conformational analog and eliminate the chiral centers is isosteric replacement of the phenyltetralin ring of sertraline and phenylpiperidine ring of paroxetine by phenylquinoxaline and phenylpiperazine, respectively, while maintaining the halogen substitution, which is essential for maintaining SSRI pharmacological activity.¹⁴ Other reports showed that positions 6 and 7 of sertraline and the equivalent position 3 of paroxetine can tolerate bulkier polar electron withdrawing groups such as carboxamide while maintaining their SSRI pharmacological activity.¹⁴ On the other hand, the main nucleus of Fluoxetine, which is another known SSRI, is benzyloxy halogenated benzene (Fig. 3).¹³ Similarly, the main nucleus of citalopram is benzyloxymethyl halogenated benzene (Fig. 3). Results show that the side chain of aliphatic amine might not be a requirement for SSRI activity since amine chains with different length (ethane, propane, butane) have shown SSRI activity but the basic nitrogen is a requirement for SSRI activity.¹³ Based on these studies, we believe that both phenylquinoxaline ring with carboxamide in position 7 and benzyloxy halogenated benzene represent promising molecular scaffolds for conferring SSRI pharmacological activity in our 'hybrid' molecules (blue groups in Figs. 3 and 4).

Design virtual hybrid SSRIs with dual action

Tethering technique simply link two molecular scaffolds believed to be responsible of the desired pharmacological effect through an appropriate covalent linker with the assumption that the linker is easily metabolized inside the body into its two components. Accordingly, mix and match the four previously described molecular scaffolds (red and blue groups, Figs. 2 and 3) will result in minimum of four different series of virtual hybrid compounds (series A–D; Fig. 4).

The selection of promising compounds for chemical synthesis and subsequent in vitro biological evaluation from the generated virtual hybrid library required a computerized cutoff value. This led us to generate pharmacophore models using the existing SSRIs from the literature as well as our proposed SSRIs with dual action to gain further insights and help determine the selection of the compounds to the next phase.

Common pharmacophore features

Sixteen compounds (Fig. 5) were collected from the literature and used as a training set in the pharmacophore building. Criteria for compound selection include diverse molecular structure and being tested using the same binding assay. Wide range of binding affinities (K_i) was chosen, ranging from 0.013 to 5000 nM. Structural information from the training set identified a set of features crucial for activity and was considered to represent a pharmacophore hypothesis. Based on previous experience¹⁵, *HypoGen* module in Discovery Studios *DS* 2.0^{16,17} was used to generate our pharmacophore models wherein it evaluates a collection of conformational models for all compounds, and maps them to the selected crucial features.

The top ranked pharmacophore model is expected to identify the common binding features and the hypothetical orientation of the active compounds interacting with their target. Our model is represented by two hydrophobic centers (Hydrophobic 1, Hydro-phobic 2; cyan color) and one hydrogen bond donor (HBD; purple color) associated to its protein acceptor site and acceptor atom (Fig. 6). The interfeature distances were considered to be 9.93, 10.59 and 4.03 Å for distances between the hydrophobic center 2 and the hydrogen bond donor, the hydrophobic center 2 and the hydrophobic center 1, the hydrophobic center 1 and hydrogen bond donor, respectively (Fig. 6).

Only one angle constraint was used for the hydrophobic and the donor atom features, thus allowing the hydrophobic centers to cover a larger domain. Since not all proposed hybrid compounds place hydrophobes in both regions, a partial match directive was used on the query for the hydrophobic centers to match compounds that contain only one.

Addition of exclusion volumes

Although ligand-based pharmacophores serve as excellent tools to probe ligand/macromolecule recognition and can serve as useful 3D-QSAR models and 3D search queries, they suffer from a major drawback: They lack steric constraints necessary to define the size of the binding pocket. This liability renders pharmacophoric models rather

promiscuous. Therefore, we decided to complement our selected pharmacophore model with exclusion spheres. Excluded volumes resemble sterically inaccessible regions within the binding site. HipHop-Refine requires a list of inactive training compounds (Fig. 7) together with two qualitative descriptors that characterize the way by which each training compound contributes in defining the exclusion space (Principal and MaxOmit-Feat).^{18,19} All the nine inactive compounds, which used in adding the steric volumes together with their HipHop-Refine parameters, have 0 as their principal value and 2 as their maximum omitted features. Figure 8 shows the final pharmacophore with 68 added exclusion volumes. Using this generated pharmacophore model, we were able to map our proposed hybrid anti-autism compounds into the model to locate the subset of promising compounds that are capable of binding to SERT with a similar set of interactions. Finally, the proposed compounds with fit values (2) were selected for chemical synthesis and biological evaluation (Table 1). Figure 9, 10 and 11 demonstrate the mapping of compounds RHO-003, RHO-004 and RHO-0012 to the generated pharmacophore with fit values 2.10, 2.30 and 1.98 respectively.

Results

Proposed compounds that met the pharmacophore criteria (Fit Value 2) were synthesized as candidates for pharmacological assessment. Scheme 1 and Scheme 2 represent the successful synthetic pathways to synthesize compounds RHO-001-RHO-012. In Scheme 1, RHO-001 was obtained by coupling 4-trifluorophenol with 2-bromo-2-phenylacetate in the presence of an inorganic base. Hydrolysis of the methyl ester of RHO-001 yielded the free carboxylic acid RHO-002, which was then subjected to couple with different primary amines in the presence of EDC/HoBt to afford the target compounds RHO-003 to RHO-005 in good yields (71–82%).

In Scheme 2, 4-fluoro-3-nitrobenzoic acid was converted to the corresponding ester RHO-006, which was then subjected to Pd-catalyzed coupling reaction with 3,4-dichloroaniline to give RHO-007. Reduction of the nitro group following by reaction with 2-chloroacetyl chloride yielded the open ring analog of RHO-009, which was then cyclized to the corresponding quinoxalinone ring RHO-010. Methylation of the cyclic amide gave RHO-011, which was then hydrolysed to the free carboxylic acid, converted to its acid chloride analog and finally couple with 4-methoxy-3(4-methylpiperazin-1-yl)aniline to afford our final target compound RHO-012 in 56% yield.²⁰

Although structural requirements for ligand binding at SERT are highly variable, a basic amine moiety usually is present among ligands with high-affinity for aminergic GPCRs—it is proposed that the protonated amine can interact with the fully-conserved aspartate residue at GPCR position 3.32.^{21,22} Accordingly, analog RHO-012 was chosen to undergo preliminary pharmacological assessment for affinity at human recombinant 5-HT_{1B} and 5-HT_{1D} GPCRs expressed in HEK cell membranes.²³ The radioreceptor competition displacement assay used [³H]-CT (carboxytryptamine) and [³H]-GR125743 to label 5-HT_{1B} and 5-HT_{1D} receptors, respectively. Figure 12 shows competition displacement curves for RHO-012 in comparison to the reference ligand ergotamine at 5-HT_{1B} and 5-HT_{1D} receptors; the *K_i* value for RHO-012 was 44 ± 7.2 and 120 ± 14 nM at 5-HT_{1B} and 5-HT_{1D} receptors, respectively. SERT binding affinity is in progress.

Conclusion

A library of virtual hybrid SSRI with dual action was designed. Pharmacophore model was generated using structurally diverse existing SSRIs with K_i range from 0.013–5000 nM. Exclusion volumes were added to the chosen model to sterically refine it. The sterically-refined version of the pharmacophore was generated and used as 3D query for compound selection. Proposed compounds with high fit values (> 2) were selected for synthesis and in vitro biological evaluation. Preliminary in vitro evaluation data is promising and consistent with our prediction. All compounds are undergoing further testing now and the full biological data will be published in a future communication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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20. Synthesis of RHO-012 A mixture of RHO-011 (200 mg, 0.55 mmol) and NaOH (440 mg, 11.0 mmol) in a mixed solvent of CH₃OH and H₂O (20 mL, 4:1 v/v) was heated under reflux for 1 h. The reaction mixture was then filtered to remove any insoluble impurities, and the resulted filtrate was concentrated in vacuo, diluted with water (20 mL) and then neutralized with 50% HCl to pH 6. The resulted precipitate was filtered, washed with cold water and left to dry in air. The dried solids were dissolved in dichloromethane (20 mL), and to the resulted solution was added thionyl chloride (0.08 mL, 1.1 mmol) in a dropwise manner. The resulted mixture was heated under reflux for 24 h, after which, the solvent and excess thionyl chloride were removed under vacuum. The residue was taken into THF (4 mL), and to this new solution was added 4-methoxy-3-(4-methylpiperazin-1-yl)aniline (146 mg, 0.66 mg), pyridine (1 mL) and a catalytic amount of DMAP, and the resulted mixture was heated at 60 °C for 20 h. The reaction solvent was then removed under vacuum, and the residue was purified by column chromatography (silica gel, ethyl acetate/acetone 8:1 v/v) to give the pure product **RHO-012** (190 mg, 56%), mp 123–124 °C; ¹H NMR (CD₃OD): δ 2.35 (s, 3H, CH₃), 2.63 (br s, 4H, 2CH₂), 3.07 (br s, 4H, 2CH₂), 3.42 (s, 3H, CH₃), 3.83 (s, 3H, CH₃), 4.24 (s, 2H, CH₂), 6.85–6.93 (m, 2H, ArH), 7.14 (dd, *J* = 2.3, 8.7 Hz, 1H, ArH), 7.29–7.37 (m, 3H, ArH), 7.45 (d, *J* = 8.6 Hz, 1H, ArH), 7.53 (d, *J* = 8.5 Hz, ArH), 7.69 (s, 1H, ArH), 7.90 (s, 1H, ArH); ¹³C NMR (CD₃OD): 28.24, 44.71, 49.84, 51.80, 54.68, 54.90, 111.53, 112.19, 114.73, 115.18, 115.81, 121.91, 123.10, 123.67, 127.20, 127.61, 130.63, 130.96, 132.02, 132.81, 135.56, 136.94, 140.82, 143.33, 149.34, 165.84.
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Figure 1.

The mechanism of action of SSRIs in the presence/absence of autoreceptor antagonists. The green arrows show the two pathways regulating serotonin (5-HT) concentration in the synaptic cleft through the reuptake transporter and autoreceptor. In panel A, administration of SSRIs alone allows the autoreceptors to inhibit 5-HT release, which delays the therapeutic effect of SSRIs. In panel B, co-administration of SSRIs and autoreceptor antagonists abolishes the inhibitory effect of the autoreceptors and synergistically increase the therapeutic effect of SSRIs.

Figure 2.
Chemical structures of known 5-HT_{1B/1D} antagonists.

Figure 3.
Chemical structures of known 5-HT reuptake inhibitors.

Figure 4.
Representative examples of virtual hybrid SSRI with dual action.

Figure 5.
Literature based SSRIs used as training set in building the pharmacophore model.

Figure 6.

Pharmacophore model used in the selection of the virtual hybrid compounds includes two hydrophobic centers (cyan color) and one hydrogen bond donor (HBD; purple color).

Figure 7.
Inactive SSRI compounds used to add exclusion spheres to the pharmacophore model.

Figure 8.
Sterically-refined versions of our pharmacophore with 68 added exclusion volumes model.

Figure 9. Mapping compound RHO-003 to the sterically-refined versions of our pharmacophore model (Fit Value = 2.10).

Figure 10. Mapping compound RHO-004 to the sterically-refined versions of our pharmacophore model (Fit Value= 2.30).

Figure 11.
Mapping compound RHO-012 to the sterically-refined versions of our pharmacophore model (Fit Value = 1.98).

Scheme 1.

Reagents and conditions: (a) K_2CO_3 , DCM, Reflux, 6 h; (b) NaOH, MeOH, rt, 2 h; (c) EDCI, HoBT, DCM, rt, 24 h.

Scheme 2.

Reagents and conditions: **(a)** SOCl_2 , MeOH, rt, 24 h; **(b)** $\text{Cl}_2(\text{PPh}_3)_2\text{Pd(II)}$, Xantphos, K_2CO_3 , Toluene, N_2 , reflux, 12 h; **(c)** Pd/C, H_2 , THF, rt, 4 h; **(d)** TEA, Dichloroethane, rt, 3 h; **(e)** KI, DBU, butanone, reflux, 4 h; **(f)** CH_3I , NaH, DMA, N_2 , rt, 24 h; **(g)** NaOH, MeOH/ H_2O (4:1), reflux, 2 h; **(h)** SOCl_2 , DCM, reflux, 24 h; **(i)** 4-methoxy-3-(4-methylpiperazin-1-yl)aniline, pyridine, DMAP, 60° , 20 h.

Figure 12.

Representative concentration-response curves for RHO-012 displacement of radioligand from human 5-HT_{1B} (panel A) and 5-HT_{1D} (panel B) receptors. Displacement by the reference ligand ergotamine is shown for comparison.

Table 1

Some proposed compounds and their Fit Values

Compound ID	Compound	Fit Value
RHO-001		Not mapped
RHO-002		Not mapped
RHO-003		2.100
RHO-004		2.304
RHO-005		2.208
RHO-010		2.212
RHO-011		Not mapped
RHO-012		1.979