Selective Serotonin Reuptake Inhibitors (SSRIs) with Dual Functionality; Hybrid Anti-Autism Candidates

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Abstract: Autism is a lifelong neurodevelopmental disorder significantly on the rise worldwide. Prevalence rate increased dramatically from 1 case/10000 in the early 90's to 1 case/110 in 2011. The diagnosis is characterized by three main domains: dysfunction in social interaction, communication impairment and repetitive behaviors. Selective Serotonin Reuptake Inhibitors (SSRIs) are currently the drug class of choice for treating autism symptoms. The major drawback of SSRIs is that they take several weeks to become therapeutically efficient. Co-administration of SSRIs with 5-$HT_{1B/1D}$ receptor antagonists proved to be advantageous over SSRIs alone with respect to the magnitude of brain serotonin levels produced. The theory is that the acute blockade of 5-$HT_{1B/1D}$ receptor would prevent the negative feedback these autoreceptors exert normally on serotonin release and hence enhance the efficiency of SSRIs (i.e. synergistic effect).

We hypothesize to incorporate the dual pharmacophoric profile of serotonin reuptake inhibition and 5-$HT_{1B/1D}$ antagonism in one single molecule carrying dual functionalities. A library of 12 virtual hybrids was successfully designed. The organic synthesis of two chosen hybrids was completed with full structure elucidation, including elemental analysis and proton-Nuclear Magnetic Resonance.

The main outcome of the study is obtaining an unprecedented library of novel hybrid molecules combining serotonin reuptake inhibition with $5HT_{1B/1D}$ antagonism in one single molecule. In addition, establishing chemical synthesis and other foundation materials needed for further investigation (*In vitro* and *in vivo* pharmacological evaluation) towards our ultimate goal of developing new therapeutic line of autism, where no other lines of treatment have been consistently successful.

Keywords: Autism, Autoreceptors, Dual Functionality, Hybrid Molecules, Serotonin, Selective Serotonin Reuptake Inhibitors, Serotonin 5-HT1B/1D antagonist.

1. BACKGROUND AND SIGNIFICANCE

Autism is a lifelong complex neurodevelopmental disorder significantly on the rise worldwide. Prevalence rate increased dramatically from 1 case per 10000 children in the early 90's to 1 case per 110 in 2011 as estimated by the National Survey of Children's health [1]. Nearly 300,000 Children aged 4-17 had received a diagnosis of autism in USA alone in 2004. There are no confirmed statistics about the number of children with autism in Qatar. However, the previous statistics set an alarming call about the number of individuals with autism in Qatar either already diagnosed or yet to be diagnosed.

The American Psychiatric Association in 2000, defined autism as a disorder that affects the development of young children [2]. The diagnosis is characterized by three main domains: (i) Dysfunction in Social interaction (ii) Impairment in language and Communication (iii) Repetitive behaviors and restricted interests. The last domain includes obsession towards particular object(s), phrase(s), and/or body movement(s) e.g. rocking head back and forth, or flapping hands. It also involves the very strict adherence to a rigid routine schedule which might manifest in the food they eat (only food with a specific color or texture), the clothes they wear (only clothes made of specific fabrics), etc. Frequency and severity of the repetitive behavior vary from one patient to another. Consequently, repetitive behaviors domain significantly interferes with simplest tasks of daily living, educational and social learning, and negatively affect the individual's quality of life and relationship with others. Until now, no primary drug has been consistently effective in treating repetitive behaviors.

Selective Serotonin Reuptake Inhibitors (SSRIs) are currently the drug class of choice for treating autism symptoms including repetitive behaviors. SSRIs are originally antidepressant agents with established efficacy for the management of depression, anxiety, obsessive compulsive disorder and recently employed for treating repetitive behavior symptoms in autistic children [3]. On one hand, a supportive body of evidence indicates that SSRIs are capable of reducing or modulating symptoms of repetitive behaviors in autistic patients although the exact mechanism might not be quite clear [3,4]. On the other hand, and as major drawback, it takes 4-6 weeks for an SSRI to become therapeutically efficient. This drawback/delay in efficiency plus other potential side effects notably irritability and insomnia vary from one patient to another and significantly decrease patient compliance, and increase anxiety level. Furthermore, the delay adds more pressure and confusion to

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also synthesized as outline in the following synthetic scheme.

3.2.1. Synthetic Scheme

3.2.2. Experimental

General Methods

All reactions were performed under an Argon atmosphere. Solvent for extraction: ACS grade. Solvent for reaction: reagent grade. Reagents: Acros, Fluka or Aldrich, highest quality available. TLC: silica gel 60 F_{254} aluminum plates (Watman); visualization by UV absorption. Flash column chromatography was performed on Sigma-Aldrich silica gel 60 (230-440 mesh). Elemental analysis (C,H,N) was performed in the Central Laboratory Unit, Qatar University. The ^{1}H- NMR spectra were recorded at 400 MHz (Bruker, Model: Advance) at Texas A&M University-Qatar; δ values are given in ppm (TMS as internal standard) and *J* values in Hz. Mass spectra were performed with Shimatzu LC/MS using electrospray ionization (ESI) and MH^+ is reported.

Methyl 2-phenyl-2-(4-(trifluoromethyl)phenoxy)acetate (3)

To a solution of 4-trifluoromethyl phenol **(1)** (0.356 g, 0.0022 mol) in dichloromethane anhydrous (10 ml) was added anhydrous K_2CO_3 (0.604 g, 0.0044 mol), methyl α-bromophenylacetate **(2)** (0.503 g [0.347 ml], 0.0022 mol). The reaction mixture was stirred at reflux temperature for 6 hours then cooled to room temperature. The reaction mixture was monitored by TLC. After completion of the reaction was indicated by TLC, K_2CO_3 was removed by filtration and the solvent was evaporated under reduced pressure, the crude residue (0.224 g) was then purified by column chromatography using hexane and ethyl acetate (90:10) as elution solvent. Compound (3) was afforded as a white powder (130mg, 20% yield). ^{1}H- NMR ($CDCl_3$, 400 MHz); δ 7.580-7.595 (dd, 2H, J=6 Hz), 7.550-7.573 (d, 2H, J=9.2 Hz), 7.425-7.440 (d, 3H, J=6 Hz), 7.015-7.047 (d, 2H, J=12.8 Hz), 5.703 (s, 1H), 3.777 (s, 3H). MS (ESI, Neg) m/z 309 (M^-). Anal. Calcd for $C_{16}H_{13}F_3O_3$: C, 61.94; H, 4.22; O; 15.47. Found: C, 62.22; H, 4.32. *(Compound RHO-003-Supporting material).*

2-Phenyl-2-(4-(trifluoromethyl)phenoxy)acetic acid (4)

To a solution of methyl 2-phenyl-2-(4-(trifluoromethyl)phenoxy)acetate **(3)** (0.130 g , 0.0004 mol) in methanol (5 ml), a solution of NaOH (0.064 g, 0.0016 mol) in 0.5 ml water was added drop wise at room temperature, immediate yellowish coloration appears. The reaction mixture was stirred for 2 hours at room temperature. Reaction was monitored by TLC. After completion of the reaction is indicated by TLC, PH of the reaction mixture was adjusted to 6-7 with 2 N HCl. Evaporation of the excess solvent was performed under reduced pressure. The excess inorganic was filtered off after dissolving the crude residue in dichloromethane/methanol mixture, and then the filtrate was evaporated under reduced pressure. The target compound was afforded as a white powder (90 mg, 76 % yield). MS (ESI, Neg) m/z 295.07 (M^-). Anal. Calcd for $C_{15}H_{11}F_3O_3$: C, 60.82; H, 3.74; O; 16.20. Found: C, 60.47; H, 3.69.

2-Phenyl-2-(4-(trifluoromethyl)phenoxy)acetic acid chloride (5)

To a solution of 2-phenyl-2-(4-(trifluoromethyl)phenoxy)acetic acid **(4)** (0.088 g, 0.0003 mol) in anhydous dichloromethane (5ml) + Dimethylformamide (0.1 ml), was added thionyl chloride (0.177 g [0.11 ml], 0.0015 mol) at room temperature. The reaction mixture was stirred at room temperature for 2 hours, yellowish coloration appears. The reaction was monitored by TLC. After completion of the reaction is indicated by TLC, the excess solvent was performed under reduced pressure to afford yellowish solid (92 mg, 98% yield) used without further purification to the next step.

2-Phenyl-1-(4-(o-tolyl)piperazin-1-yl)-2-(4-(trifluoromethyl)phenoxy)ethanone(6)

A mixture of *o*-tolylpiperazine HCl (0.063g, 0.0003 mol) and triethylamine (0.152 g [0.10 ml], 0.0015 mol) in anhydrous dichloromethane (4 ml) was stirred at room temperature for 15 minutes. Then, a solution of 2-phenyl-2-(4-(trifluoromethyl) phenoxy)acetic acid chloride **(5)** (0.092 g, 0.0003 mol) in anhydrous dichloro-methane (1 ml) was added. The reaction mixture was stirred at room temperature overnight (14 hours). The reaction was monitored by TLC. After completion of the reaction is indicated by TLC, the reaction mixture was diluted with dichloromethane (20 ml) and sequentially washed with water, saturated $NaHCO_3$ solution and brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude residue was then purified by column chromatography using hexane and ethyl acetate (95:05) as elution solvent. The target compound was afforded as yellow oil (70mg, 51.4% yield). ^{1}H-NMR (CD_3OD, 400 MHz); δ 7.596-7.643 (m, 4H), 7.470-7.490 (m, 3H), 7.140-7.184 (m, 3H), 7.085-7.130 (t, 1H, J= 10.8, 7.2 Hz), 6.940-6.985 (t, 1H, J=10.8, 7.2 Hz), 6.884-6.903 (d, 1H, J= 7.6 Hz), 6.332 (s, 1H), 3.750-3.900 (m, 3H), 3.650-3.750 (m, 1H), 2.770-2.920 (m, 3H), 2.460-2.520 (m, 1H), 2.288 (s, 3H), MS (ESI, Pos) m/z 455 (M^+). *(Compound RHO-006-supporting material).*

2-Phenyl-N-(pyridin-3-ylmethyl)-2-(4-(trifluoromethyl)phenoxy)acetamide (7)

A mixture of 2-phenyl-2-(4-(trifluoromethyl)phenoxy) acetic acid **(4)** (0.088 g, 0.0003 mol), N-(3-Dimethyl-aminopropyl)-N′-ethylcarbodiimide hydrochloride (0.058 g, 0.0003 mol), and 1-Hydroxybenzotriazole hydrate (HOBt) (0.041 g, 0.0003 mol) in anhydrous dichloromethane (1 ml) was stirred at room temperature for 15 min. To this solution, 3-(aminomethyl) pyridine (0.032 g, 0.00033 mol) was added. Immediate white precipitation appears. The reaction mixture was stirred at room temperature for 24 hours). The reaction was monitored by TLC. After completion of the reaction is indicated by TLC, the reaction mixture was diluted with dichloromethane (10 ml) and sequentially washed with water, saturated $NaHCO_3$ solution and brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude residue was then purified by column chromatography using dichloromethane and methanol (90:10) as elution solvent. The target compound was afforded as yellow powder (90mg, 78.2% yield). ^{1}H-NMR (CD_3OD, 400 MHz); δ 8.402 (s, 2H), 7.550-7.628 (m, 5H), 7.372-7.438 (m, 3H), 7.288-7.330 (m, 1H), 7.149-7.171 (d, 2H, J=8.8 Hz), 5.797 (s, 1H), 4.457 (s, 2H), MS (ESI, Pos) m/z 387 (M^+). *(Compound RHO-007-supporting material).*

4. CONCLUSIONS AND OUTCOMES

4.1. Our research study supplies an unprecedented library of novel virtual hybrid compounds combining the dual pharmacophoric profile of 5-HT reuptake inhibition and 5HT1B/1D antagonism as a potential developer of fast acting SSRI and as anti-autism candidate.

4.2. Our study provides two novel synthesized hybrid compounds for further biological evaluation (*in vitro* and *in vivo*).

4.3. Our work herein establishes the foundation material necessary towards our ultimate goal of developing new therapeutic line of treatment for autism, where no other lines of treatment have been consistently successful.

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