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Synthesis and biological evaluation of the major metabolite of atomoxetine: elucidation of a partial κ-opioid agonist effect

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Abstract—The major human metabolite of atomoxetine (4-hydroxyatomoxetine) was tested against a panel of receptors and enzymes, and was found to interact with the μ, δ, and κ-opioid receptors based upon studies involving both binding and functional assays. 4-Hydroxyatomoxetine was determined to be a partial agonist of the κ-opioid receptor.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is characterized by three core symptoms: inattention, hyperactivity, and impulsivity. This neuropsychiatric condition often has lifelong consequences with significant negative impact on educational, occupational, and social outcomes. ADHD is the most commonly diagnosed childhood behavioral disorder, affecting approximately 3–5% of the school-age population. ADHD is a heterogeneous disorder with diverse etiological origins, clinical manifestations, and levels of severity.[1]

Stimulants are the mainstay of pharmacological treatment for ADHD and have been effectively used for over 50 years. This group of medications includes methylphenidate (**1**), amphetamines (**2**), and pemoline (**3**). Methylphenidate is the most commonly prescribed medication for ADHD.

Although stimulants are often effective in the treatment of ADHD, some patients do not adequately respond to this treatment paradigm.[2] Additionally, these compounds are short acting resulting in the need for multiple dosing throughout the day. A once-daily regimen is preferred in order to minimize side-effects and increase patient compliance.[3,4] This dilemma has recently been resolved by the development of several

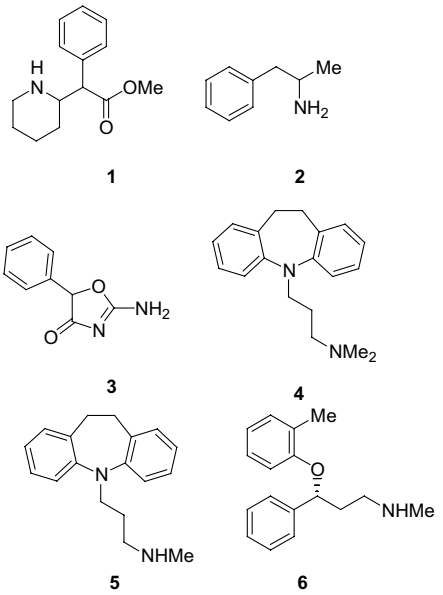

Figure 1. Structures of methylphenidate (**1**), amphetamine (**2**), pemoline (**3**), imipramine (**4**), desipramine (**5**), and atomoxetine (**6**).

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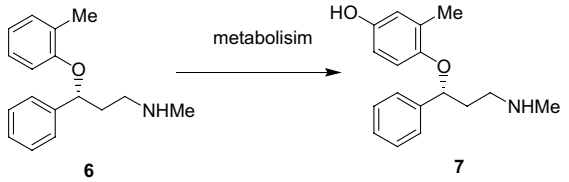
effective long-acting formulations. For example, an osmotically-controlled extended-release form of methylphenidate has been developed, which overcomes tachyphylaxis and provides effective long acting treatment, without abuse potential.[5]

Tricyclic antidepressants have also been applied as alternative medications for ADHD, but the results are inconsistent and sometimes tragic. The use of imipramine (**4**) and desipramine (**5**), the most frequently prescribed antidepressants for the treatment of ADHD, is currently clouded by concerns about their safety.[6] Therefore, while efficacious, these compounds are considered second-line agents for the treatment of ADHD.

Atomoxetine (**6**) has been recently approved for the treatment of ADHD. Atomoxetine is structurally related to fluoxetine yet selectively blocks the presynaptic norepinephrine transporter causing a measurable increase in extracellular levels of norepinephrine and dopamine in the prefrontal cortex.[7–9] This compound may represent a new paradigm for the treatment of ADHD. We are currently interested in understanding the pharmacology of selective and mixed monoamine transport inhibitors for the treatment of a variety of CNS disorders. It has been reported that atomoxetine (**6**) undergoes oxidative metabolism in humans leading to the formation of a major phase I metabolite, 4-hydroxyatomoxetine (**7**), and a minor metabolite resulting from demethylation, N-desmethylatomoxetine (Scheme 1).[10] The primary enzyme responsible for the formation of **7** from **6** has been identified as cytochrome P450 CYP2D6. We identified **7** as the major component upon treatment of **6** with human liver microsomes, prepared **7** synthetically, and evaluated it against a panel of receptors, ion channels, and enzymes. We found that compound **7** significantly interacted with the μ, δ, and κ-opioid receptors at a concentration of 10 μM. Therefore we examined the pharmacological properties of **7** at opioid receptors in more detail using binding and functional assays.

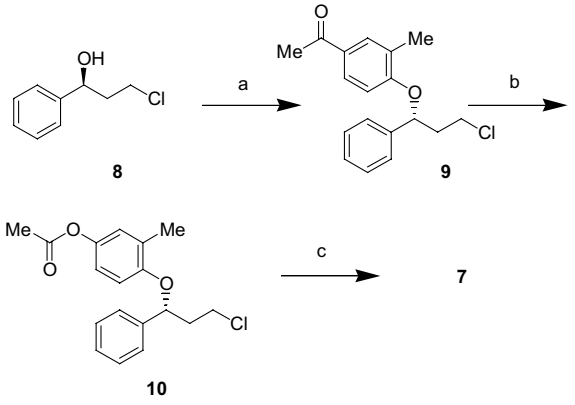
2. Chemistry

The synthesis of **7** was initiated by Mitsunobu condensation of 4-hydroxy-3-methylacetophenone with (*S*)-3-chloro-1-phenyl-1-propanol **8** to provide ether **9** (Scheme 2). Baeyer–Villiger rearrangement of **9** yielded ester **10**, which was subsequently treated with methylamine in a water/ethanol mixture at 150 °C to afford the desired **7** in approximately 50% yield for the three steps (Fig. 1).



6 **7**

Scheme 1.



Scheme 2. Reagents and conditions: (a) DEAD, PPh_3, 4-hydroxy-3-methylacetophenone; (b) *m*CPBA, $CHCl_3$, reflux; (c) EtOH, $MeNH_2$ (40% in water), 150 °C, 10 min.

3. Biology

HEK-293 cell membranes from cells stably expressing the κ-opioid receptor and other opioid receptors were prepared as described, with the exception that the binding buffer used was 50 mM Tris–Cl pH 7.8, 5 mM $MgCl_2$ and 1 mM EGTA.[11]

The κ-opioid and other opioid receptors are coupled to the heterotrimeric G protein G_i, and do not normally elicit a calcium response. Therefore, stable cell lines expressing the respective opioid receptors and the G_{qi5} construct (molecular devices) were generated. The G_{qi5} construct expresses a hybrid G_q/G_i protein that redirects G_i signaling to the G_q pathway, leading to calcium release. HEK-293 cells expressing receptor and G_{qi5} were plated onto 96-well plates at a density of 50,000 cells/well in a total volume of 50 μL. Two days later cells were prepared for assay using the FLIPR Calcium Assay Kit (molecular devices) according to manufacturer's directions, with the exception that the volume of dye mix added to each well was 50 μL instead of 100 μL. Cells were treated with compound at the indicated concentrations, added in a total volume of 100 μL at twofold the final concentration. Data points were collected at one per second for 120 s, then one every three seconds for 30 s for a total collection time of 150 s. The data were used to generate EC_{50} curves using GraphPad Prizm v3.0, and the data for the κ-opioid receptor is shown in Figure 2.

4. Discussion

Biological screening against the three opioid receptors shown in Table 1 indicated that 4-hydroxyatomoxetine (**7**) binds to both the μ and κ-opioid receptors to the extent of 164 and 88 nM IC_{50}'s, respectively. The values for the μ and κ-opioid receptors for **7** were 25- and 50-fold greater than those corresponding to the parent **6**. Subsequent analysis of the pharmacological function of **7** (Fig. 2) on these receptors showed that 4-hydroxyatomoxetine is a partial agonist for the κ-opioid receptor,

Table 1. Binding of atomoxetine (**6**) and 4-hydroxyatomoxetine (**7**) to opioid receptors (IC_{50} values, nM)

Compound	Mu	Delta	Kappa	ORL-1
6	4186	>5000	4383	>5000
7	164	1490	88	>5000

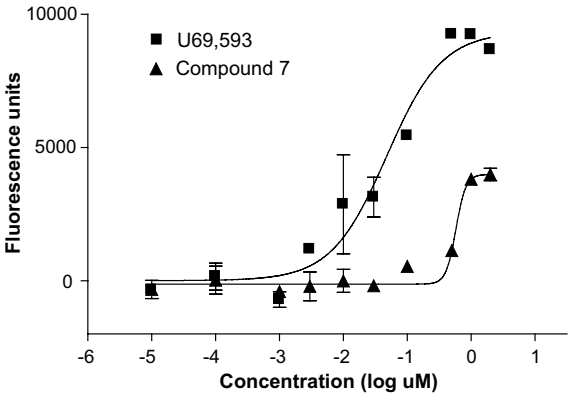


Figure 2. Concentration response curves for 4-hydroxyatomoxetine (**7**) and U69,593 (a κ agonist).

while we observed no activation of the μ-receptor (data not shown). The partial κ-opioid agonism of 4-hydroxyatomoxetine (**7**) may have a clinical correlate upon long-term exposure of atomoxetine (**6**) in the treatment of ADHD. It is known that prolonged activation of κ-opioid receptors due to chronic administration of a κ-opioid agonist results in the development of physical dependence and withdrawal symptoms upon cessation.[12] Also, κ-opioid agonists are known to cause CNS-related adverse events, most notably dysphoria.[13]

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