Pharmacological properties and discriminative stimulus effects of a novel and selective 5-HT2 receptor agonist AL-38022A
[(S)-2-(8,9-dihydro-7H-pyran[2,3-g]indazol-1-yl)-1-methylethylamine]

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Abstract
AL-38022A is a novel synthetic serotoninergic (5-HT) ligand that exhibited high affinity for each of the 5-HT2 receptor subtypes (K i ≤ 2.2 nM), but a significantly lower (~100-fold less) affinity for other 5-HT receptors. In addition, AL-38022A displayed a very low affinity for a broad array of other receptors, neurotransmitter transport sites, ion channels, and second messenger elements, making it a relatively selective agent. AL-38022A potently stimulated functional responses via native and cloned rat (EC50 range: 1.9–22.5 nM) and human (EC50 range: 0.5–2.2 nM) 5-HT2 receptor subtypes including [Ca2+] i mobilization and tissue contractions with apparently similar potencies and intrinsic activities and was a full agonist at all 5-HT2 receptor subtypes. The CNS activity of AL-38022A was assessed by evaluating its discriminative stimulus effects in both a rat and a monkey drug discrimination paradigm using DOM as the training drug. AL-38022A fully generalized to the DOM stimulus in each of these studies; in monkeys MDL 100907 antagonized both DOM and AL-38022A. The pharmacological profile of AL-38022A suggests that it could be a useful tool in defining 5-HT2 receptor signaling and receptor characterization where 5-HT may function as a neurotransmitter.

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1. Introduction
Activation of serotonin-2A (5-HT2A) receptors in the brain has been demonstrated to have a major role in affecting the centrally mediated behavioral responses observed for ergolines (e.g., d-lysergic acid diethylamide), phenylethylamines (e.g., mescaline and 2,5-dimethoxy-4-methylphenylisopropylamine, DOM), and indolealkylamines (e.g., bufotenine and N,N-dimethyltryptamine) (Glennon, 1996). Many studies have been conducted to gain insight into the critical molecular events associated with agonist binding at the 5-HT2A receptor and how this association might initiate the requisite signaling cascades to elicit a behavioral response, but these mechanisms remain poorly understood. Different binding orientations have been proposed for partial agonists and full agonists at the 5-HT2A receptor (Ebersole et al., 2003; Shapiro et al., 2000; Weinstein, 2006) suggesting that dissimilar ligand-induced active conformations of the receptor can initiate productive G-protein activation, which in turn can then be propagated through similar or different signaling cascades. Similar differential binding of agonists and partial agonists has been observed for other GPCRs, such as the β2-adrenergic and cholecystokinin-1 receptors (Archer-Lahlou et al., 2005; Ghanouni et al., 2001; Swaminath et al., 2005). Moreover, the realization of agonist-directed trafficking of receptor stimulus (functional selectivity) by agonists of 5-HT2 receptors, that is, potential activation of multiple signal transduction pathways via inositol phosphates/diacylglycerol formation, arachidonic acid release, 2-arachidonoylglycerol formation, and phospholipase D activation (Berg et al., 1998; Parrish and Nichols, 2006), illustrates the considerable challenge to defining the complex array of events involved in 5-HT2 receptor agonist-mediated induction of behavioral responses.

Within the phenylethylamine class of psychoactive agents many of the 4-substituted 2,5-dimethoxyphenylethylamine (DOX) analogs, some of which are partial agonists at the 5-HT2A receptor, have shown a pronounced hallucinogenic response in man (Shulgin and Shulgin, 1991). The DOX class of compounds has been well studied due to the selectivity of these agents for the 5-HT2 receptor, with no significant agonist activity at the 5-HT1A receptor or any significant affinity for other receptors, unlike tryptamine analogs which have...
affinity for and agonist activity at multiple receptors (Glennon, 1996; Nichols, 2004). Furthermore, the commercial availability of the non-scheduled 4-iodo analog (DOI) has made it the current molecule of choice for investigations wherein a selective 5-HT2 agonist is desired. However, as noted above, DOI activation of 5-HT2 receptors may not be representative of the receptor activation and subsequent signal transduction utilized by full agonists of other chemical classes. Therefore, additional compounds, particularly indoleamine analogs, that are selective full agonists at the 5-HT2 receptors, could prove to be useful for gaining a more complete understanding of agonist-receptor interactions in conjunction with the multiple signaling pathways identified for these receptors, even if, as with the phenethylamines, they lack 5-HT2 receptor subtype selectivity.

During the course of our investigation into the role that 5-HT2 receptors might have in the development and progression of ocular hypertension and glaucoma (Glennon et al., 2004; May et al., 2003a; May et al., 2003b; May et al., 2006), wherein our interest was the identification of agonists that would not readily enter the CNS, we identified a novel compound that did readily enter the brain and displayed pronounced CNS activity. While this compound was not suitable for our specific needs for an ocular therapeutic agent, a broader pharmacological assessment was warranted to determine if its overall profile might complement that of currently available 5-HT2 receptor agonists. This pursuit was of particular interest in view of renewed interest in the clinical relevance of 5-HT2A receptor agonists, particularly their use for the treatment of a variety of conditions such as obsessive-compulsive disorder (Moreno et al., 2006) among other CNS disorders (Griffiths et al., 2006) including the study of cognition and memory. Furthermore, there is continued interest in identifying structurally novel classes of compounds that might assist in expanding the current understanding of the pharmacology and neurochemistry of such agents at the molecular level.

Herein we report the results of in vitro receptor binding and functional response studies and provide selected physicochemical properties of the indolealkylamine analog AL-38022A [(S)-2-[(8,9-dihydro-7H-pyrano[2,3-β]indazol-1-yl)-1-methylthelyalmine] (Fig. 1), which demonstrate it to be a selective 5-HT2 receptor agonist with excellent solution stability. To assess the CNS activity of AL-38022A its discriminative stimulus effects were evaluated in both a rat and a monkey drug discrimination paradigm using DOM as the training drug. AL-38022A fully generalized to the DOM stimulus in each of these studies. The pharmacological profile of AL-38022A suggests that it could be a useful tool in defining 5-HT2 receptor signaling and receptor characterization where 5-HT may function as a neurotransmitter.

2. Materials and methods

2.1. Chemicals

Serotonin hydrochloride (5-HT), N,N-dimethyl-5-methoxytryptamine (5-OMe DMT), α-methyl-5-hydroxytryptamine maleate, and R-(-)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride (R-DOI) were purchased from Sigma/RBI (St. Louis, MO). (S)-2-(8,9-dihydro-7H-pyrano[2,3-β]indazol-1-yl)-1-methylthelyalmine hydrochloride (AL-38022A) was synthesized at Alcon Research, Ltd. (Fort Worth, TX) (see Supplemental Data). For the rat drug discrimination studies, (±)-1-(2,5-dimethoxy-4-methylphenyl)-2-amino-propane hydrochloride (DOM) was a gift from NIDA, NIH (Bethesda, MD); R-DOI HCl and 5-OMe DMT hydrogen oxalate were synthesized at VCU, Richmond, VA. For the monkey drug discrimination studies DOM was synthesized at Alcon by reported procedures (Matin et al., 1974; Shulgin, 1970) and (R)-(+-α-(2,3-dimethoxyphenyl)-1-2-[4-fluorophenyl]ethyl]-4-piperidinemethanol (MDL 100907) was synthesized at the Laboratory of Medicinal Chemistry, NIDDK, NIH (Bethesda, MD) as previously described (Ullrich and Rice, 2000). MDL 100907 was dissolved in 20% aqueous dimethyl sulfoxide (v/v).

2.1.1. Determination of distribution coefficient

The compound was partitioned between 1-octanol and aqueous buffer (pH 7.4, 0.1 M phosphate). The initial concentration (C0) of compound in buffer and the buffer concentration following extraction with 1-octanol (C2) were determined by RP-HPLC analysis against concentration standards for the specific compound. The distribution coefficient (DC) of a compound at a given pH was calculated using the equation DC=([C1−C2])/C2. Each determination was run in duplicate.

2.1.2. pKa determination

The ionization constant was determined by potentiometric titration (Kyoto AT-310 Potentiometric Titrator) in water containing 10 to 40% acetonitrile. The nominal pKa values obtained for each solvent mixture were plotted against the percentage of organic solvent to provide by extrapolation the pKa of the compound in water.

2.1.3. Determination of compound stability

The aqueous stability was conducted in pH 7.4, 0.025 M sodium phosphate buffer. The compound was dissolved [1% and 5 μg/ml (0.0005%)] in buffer and the solutions were heated at 75 °C for up to 4 weeks. Water for injection was used for buffer preparation. For pH adjustment, 0.6 N HCl and 1.0 N NaOH stored in glass containers were used. An HPLC method was developed for the analysis of each compound. The stability results (percent degradation) were used for calculation of the predicted half-life of a compound at 25 °C. This prediction was based on the time required for a 10% loss of compound (T90) and the fact that the rate of degradation for a first order reaction decreases 50% for every 10 °C drop in temperature.

2.2. In vitro binding assays

2.2.1. Determination of binding to rat 5-HT2A receptors

The procedure was previously described. (May et al., 2003a). In brief, the relative affinities of compounds at the 5-HT2A receptors were determined by measuring their ability to compete for the binding of the agonist radioligand [3H]-DOI to rat brain 5-HT2A receptors. Aliquots of postmortem rat cerebral cortex homogenates (400 μl) dispersed in 50 mM Tris–HCl buffer (pH 7.4) were incubated with [3H]-DOI (80 pM final) in the absence or presence of methiothepin (10 μM final) to define total and non-specific binding, respectively, in a total volume of 0.5 ml. The assay mixture was incubated for 1 h at 23 °C in polypyrene tubes and the assays terminated by rapid vacuum filtration over Whatman GF/B glass fiber filters previously soaked in 0.3% polyethyleneimine using ice-cold buffer. The samples were counted on a γ-scintillation counter and the data analyzed using a non-linear, iterative curve-fitting computer program. (Bowen and Jerman, 1995; Sharif et al., 1991).

2.2.2. Determination of binding at cloned human 5-HT2 receptors

Binding affinity of compounds at the cloned human 5-HT2A, 5-HT2B, and 5-HT2C receptors expressed in Chinese hamster ovary cells using the agonist [3H]-DOI (0.2 nM; 15 min at 37 °C) as the radioligand for each receptor was determined and reported as Ki.
values. These studies were conducted at Cerep, Poitiers, France using radioligand binding techniques similar to those described above.

2.2.3. Determination of binding at cloned human $\alpha_{\text{2A}}$ and $\alpha_{\text{2C}}$ adrenergic receptors

The procedure was previously described (May et al., 2006). Briefly, membranes from SF9 cells expressing the cloned human $\alpha_{\text{2A}}$ or $\alpha_{\text{2C}}$ adrenergic receptor [BioSignal Packard, Inc. (Montreal, PQ, Canada)] were suspended in 75 mM Tris–HCl buffer diluted to 32 µg/ml and 48 µg/ml protein, respectively, in Tris–HCl containing 12.5 mM MgCl$_2$ and 2 mM EDTA (pH 7.4). The diluted test compound (25 µl), followed by a volume of 200 µl of receptor preparation, and finally 25 µl of [H]-clonidine (28 nM final concentration) were added to a 96 well plate. The incubations (60 min at 23 °C) were terminated by rapid vacuum filtration using Whatman GF/C glass fiber filters that were previously soaked in 0.3% polyethyleneimine. The filters were washed with ice-cold 50 mM Tris–HCl, pH 7.4. The samples were counted on a TopCount scintillation counter (Packard Instruments, Meriden, CT).

2.2.4. Determination of other receptor binding activity

Binding assays for human 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{7}$ and rat 5-HT$_{1B}$ serotonergic receptors, human $\alpha_{\text{2A}}$ and $\alpha_{\text{2C}}$ and rat $\alpha_{\text{2B}}$ adrenergic receptors, and human norepinephrine and serotonin transporters were conducted at NovaScreen Biosciences (Hanover, MD) using their standard screening protocols (Sweetnam et al., 1995).

Determination of binding affinity for human 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_{6}$ and porcine 5-HT$_{2}$ receptors, rat $\alpha_{\text{1A}}$ and $\alpha_{\text{1B}}$ adrenergic receptors, human D$_{1}$, D$_{2L}$, D$_{2S}$, D$_{4}$ and the rat D$_{3}$ receptors were conducted by means of standard radioligand binding assay techniques using the conditions summarized in Supplemental data Table S1.

2.3. In vitro functional assays

2.3.1. Determination of human 5-HT$_{1A}$ receptor activity. Inhibition of cAMP production in cultured cells

The procedure was previously described (May et al., 2003b; Sharif et al., 2004). Briefly, Chinese hamster ovary (CHO) cells expressing the cloned human 5-HT$_{1A}$ receptor [Euroscreen (Brussels, Belgium)] were pre-incubated with the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (1 mM final), for 20 min at 23 °C followed by the addition of the test compounds and the incubation continued for another 20 min. The adenylyl cyclase activator, forskolin (10 µM), was added and the incubation terminated after 10 min using ice-cold 0.1 M acetic acid. The measurement of cAMP was performed using an enzymeimmunoassay as previously described. The inhibition of forskolin-induced cAMP production by the test compounds was analyzed using a non-linear, iterative curve-fitting computer program (Bowen and Jerman, 1995; Sharif et al., 1991).

2.3.2. Determination of rat 5-HT$_{2A}$ receptor activity by [Ca$^{2+}$], mobilization assay

The procedure was previously described (Kelly et al., 2003; Sharif et al., 2006). Briefly, the receptor-mediated mobilization of intracellular calcium ([Ca$^{2+}$]) was studied with the Fluorescence Imaging Plate Reader (FLIPR) using rat vascular smooth muscle cells (A7r5, expressing native 5-HT$_{2}$ receptors) in 96-well culture plates (Kelly et al., 2003). An aliquot (25 µl) of the test compound was added to the Ca$^{2+}$-sensitive dye-loaded cells and the fluorescence data collected in real-time at 1.0 second intervals for the first 60 s and at 6.0 second intervals for an additional 120 s. Responses were measured as peak fluorescence intensity minus basal and, where appropriate, were expressed as a percentage of a maximum 5-HT-induced response ($E_{\text{max}}$). The concentration-response data were analyzed using a non-linear, iterative curve-fitting computer program (Kelly et al., 2003). Even though our previous studies have indicated primarily the presence of 5-HT$_{2A}$ receptors in the A7r5 cells, a contribution from 5-HT$_{2B}$ receptors in the overall functional response detected is possible (Watts et al., 2001).

2.3.3. Determination of rat 5-HT$_{2B}$ receptor activity by isolated rat stomach fundus assay

This assay was conducted by MDS Pharma Services, Bothell, Washington, using methods previously described (Cohen and Fludzinski, 1987). In brief, longitudinal stomach fundus strips dissected from adult Wistar rats were mounted in 25 ml organ baths containing oxygenated Krebs buffer (pH 7.4) maintained at 37 °C. After a 45 min equilibration period, 10 µl aliquots of test agents were added to the organ bath (10 ml volume) and isometric tension recorded via an FT3 transducer. Cumulative contractile dose–response curves were constructed for test agonists. Alpha-methyl-5-HT was used as a standard reference agonist. Dose–response data were analyzed as described above to obtain the potency values (EC$_{50}$) of test agents.

2.3.4. Determination of rat 5-HT$_{2C}$ receptor activity by [Ca$^{2+}$], mobilization assay

These assays were performed as for the 5-HT$_{2A}$ receptor above, except that SR373 cells expressing the recombinant rat 5-HT$_{2C}$ receptor were utilized.

2.3.5. Determination of cloned human 5-HT$_{2}$ receptor subtypes activity by aequorin based [Ca$^{2+}$] mobilization assay

Functional response at the 5-HT$_{2}$ receptor subtypes was determined using CHO-K1 cells stably expressing mitochondrial-targeted bioluminescent aequorin, C$_{\text{a}_{\text{160}}}$. and one of either human serotonin receptor clone 5-HT$_{2A}$, 5-HT$_{2B}$, or 5-HT$_{2C}$. Prior to testing, cells were loaded in suspension with coelenterazine for 4–16 h and directly injected onto different concentrations of the test compound. Light emitted from the cells was measured 20–30 s following receptor activation. A luminometer (Hamamatsu, FDSS-6000) was used to record luminescence in response to the test compound. The mean response signal at each of 8–11 different concentrations was integrated to provide an estimation of receptor activation, expressed as the EC$_{50}$ value. The efficacy of the response ($E_{\text{max}}$) at the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors is expressed relative to the response of α-methyl-5-HT under the same assay conditions while the efficacy at 5-HT$_{2C}$ is expressed relative to the response of 5-HT. These studies were conducted at Euroscreen s.a., Brussels, Belgium.

2.3.6. Determination of human $\beta_{2}$-adrenergic receptor activity

Agonist and antagonist functional activity was determined in immortalized human non-pigmented ciliary epithelial (NPE) cells that express endogenous $\beta_{2}$-adrenergic receptors as previously described (Crider and Sharif, 2002). In brief, confluent NPE cells were incubated with AL-38022A (1 nM–100 µM) for 15 min in the presence of 1 mM IBMX at 23 °C. When AL-38022A was tested as an inhibitor of $\beta_{2}$-adrenergic receptor-induced cAMP production, it was added to the cells 15 min before the addition of isoproterenol (100 nM) and the assay continued for another 10 min. The assays were terminated using ice-cold 0.1 M acetic acid and the measurement of cAMP was performed using an enzymeimmunoassay as previously described (Crider and Sharif, 2002). Data were analyzed using an iterative non-linear curve-fitting computer program (Bowen and Jerman, 1995; Sharif et al., 1991).

2.4. Experimental animals

2.4.1. Rats

Nine male Sprague–Dawley rats (Charles River Laboratories), weighing 250–300 g at the beginning of the study, were trained to discriminate (15-min pre-session injection interval) 1.0 mg/kg of DOM from vehicle (sterile 0.9% saline) under a variable interval 15-s schedule of reinforcement for appetitive reward (i.e., sweetened condensed
2.4.2. Monkeys

Four adult rhesus monkeys (two males and two females) weighing between 5 kg and 8 kg were housed individually with unlimited access to water. Diet comprised primate chow (Harlan Teklad High Protein Monkey Diet, Madison, WI), fresh fruit and peanuts. Food was provided to monkeys after daily sessions that was sufficient to maintain normal, age- and gender-appropriate weights. Monkeys were maintained on a 14-h/10-h light/dark cycle and had been trained previously to discriminate between DOM and vehicle in a standard two-lever discrimination procedure (Li et al., 2008). The animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee (Institute of Laboratory Animals Resources on Life Sciences, National Research Council, National Academy of Sciences).

2.5. Drug discrimination studies in rat

In brief, animals were food-restricted to maintain their body weights at approximately 80% of their free-feeding weight, but were allowed access to water ad lib in their individual home cages. Daily training sessions were conducted with the training dose of DOM or saline. For about half the animals, the right lever was designated as the drug-appropriate lever, whereas the situation was reversed for the remainder of the animals. Learning was assessed every fifth day during an initial 2.5-min non-reinforced (extinction) session followed by a 12.5-min training session. Data collected during the extinction session included number of responses on the drug-appropriate lever (expressed as a percent of total responses) and response rate (i.e., responses per minute). Animals were not used in the subsequent stimulus generalization studies until they consistently made ≥80% of their responses on the drug-appropriate lever after administration of training drug and ≤20% of their responses on the same drug-appropriate lever after administration of saline for several weeks. During the stimulus generalization (i.e., substitution) phase of the study, maintenance of the DOM/saline discrimination was insured by continuation of the training sessions on a daily basis (except on a generalization test day). On one of the two days before a generalization test, about half the animals received the training dose of DOM and the remainder received saline; after a 2.5-min extinction session, training was continued for 12.5 min. Animals not meeting the original training criteria during the extinction session were excluded from the immediately subsequent generalization test session. During the investigations of stimulus generalization, test sessions were interposed among the training sessions. The animals were allowed 2.5 min to respond under non-reinforcement conditions. An odd number of training sessions (usually 5) separated any two generalization test sessions. Doses of test drugs were administered in a random order. A determination of complete, partial, or no generalization was based on previously described criteria (Young and Glennon, 1986). In this scheme, the results of stimulus generalization tests are characterized as one of three possible results: a) complete stimulus generalization resulted when the animals, following a given dose of drug or drug combination, made ≥80% (i.e. group mean) of their responses on the drug-appropriate lever, b) no generalization (i.e. saline-like responding) occurred when the test agent produced 0–20% drug-appropriate responding, and c) partial generalization occurred when a challenge drug produced an intermediate level of responding (i.e. 21% to 79% but usually between 40% and 70%) on the drug-appropriate lever. Animals making fewer than 5 total responses during the 2.5-min extinction session were considered as being behaviorally disrupted. Percent drug-appropriate responding and response rate data refer only to animals making ≥5 responses during the extinction session.

DOM, saline, and the test drugs were administered 15 min prior to testing. Where stimulus generalization occurred, an ED_{50} dose was calculated by the method of Finney (Finney, 1952). The ED_{50} dose represents the drug dose where animals would be expected to make 50% of their responses on the drug-appropriate lever. Response rate data (i.e. responses/min) were evaluated by Dunnett’s t-test (p<0.05) for comparison of a control group (i.e. mean response rate after saline) versus dose(s) of training drug or experimental dose groups of a test compound. All doses of all drugs were administered via intraperitoneal injection; doses refer to the weight of the salts. Solutions in sterile 0.9% saline were freshly prepared each day.

2.6. Drug discrimination studies in rhesus monkeys

2.6.1. Apparatus

During experimental sessions, subjects were seated in chairs (Model R001, Primate Products, Miami, FL) that provided restraint at the neck and arms and were located in ventilated, sound-attenuating chambers. Each chamber was equipped with two stimulus lights and two response levers. The monkeys responded under a schedule of stimulus-shock termination (SST). The feet of monkeys were placed in shoes that were mounted to the front of the chair and equipped with brass electrodes to which a brief (250 ms, 3 mA) electric shock could be delivered from an a.c. generator. Experiments were controlled and data recorded and collected with a microprocessor and commercially available interface (Med Associates Inc., East Fairfield, VT).

2.6.2. Drug discrimination

Daily training sessions began with a 30-min timeout period, during which stimulus lights were not illuminated and responding had no programmed consequence. This timeout period was followed by a 10-min response period during which two stimulus lights were illuminated above the levers. Monkeys could extinguish stimulus lights and postpone the shock schedule for 30 s by responding five times consecutively (fixed ratio [FR] 5) on the lever designated correct by an injection administered during the first minute of the cycle (e.g., right lever, saline; left lever, DOM). Incorrect responses reset the FR requirement on the correct lever. Failure to satisfy the FR requirement within 30 s of illumination of the stimulus lights resulted in the delivery of a brief shock. Thereafter, shock was delivered every 30 s until the response requirement was satisfied, the cycle ended, or a total of four shocks was delivered, which ever occurred first.

Injections were made s.c. in the back during the first minute of the training session. All monkeys had satisfied the following criteria before this study began: at least 80% of the total responses on the correct lever; and fewer than 5 responses on the incorrect lever prior to completion of the first FR on the correct lever (Li et al., 2008). Monkeys were tested every third day provided that the testing criteria were satisfied during intervening training sessions. If a monkey failed to satisfy these criteria during one of the training sessions, training continued until the criteria were satisfied.

Test sessions were similar to training sessions except that 5 consecutive responses on either lever postponed shock and different doses of DOM, R-DOI or AL-38022A were administered during the first minute of the session. Different doses were studied in a nonsystematic order. Antagonism studies were conducted by administering MDL 100907, a highly selective 5-HT_{2A} receptor antagonist, 5 min before the administration of DOM (0.32 mg/kg) or AL-38022A (0.1 mg/kg).

2.7. Statistical analyses

The drug discrimination data from monkeys were analyzed with a two-way analyses of variance (ANOVA) for repeated measures with
one factor comprising agonist treatment (DOM and AL-38022A) and a second factor comprising MDL 100907 dose (0.00032–0.032 mg/kg; p < 0.05). A post-hoc Tukey–Kramer test was used to examine significant differences among treatments (p < 0.05).

3. Results

3.1. Physicochemical data

The distribution coefficients (DC) for R-DOI, 5-Ome DMT, and AL-38022A were determined to be 3.03, 3.30, and 4.39, respectively. The ionization constant for the basic primary amine of AL-38022A was determined to be 8.45 while that of R-DOI and 5-Ome DMT were 9.89 and 9.10, respectively. The aqueous solubility of AL-38022A was greater than 1% in phosphate buffer at pH 7.4; AL-38022A was determined to have excellent solution stability; as a dilute solution (200 µM) for completeness.

DOI has a significantly higher affinity (28-fold) does show a modest 28-fold higher affinity (3.1. Physicochemical data) for the 5-HT2A receptor relative to 5-HT; while AL-38022A at these receptors, though the functional potency of AL-38022A is comparable to that of 5-HT (0.3 nM), but approximately 10-fold higher than that of 5-HT and nearly 100-fold greater than 5-OMe DMT.

Table 2

<table>
<thead>
<tr>
<th>Kinetic Parameters</th>
<th>AL-38022A</th>
<th>R-DOI</th>
<th>5-Ome DMT</th>
<th>AL-38022A</th>
<th>R-DOI</th>
<th>5-Ome DMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>K&lt;sub&gt;i&lt;/sub&gt; (nM)</td>
<td>0.13±0.04</td>
<td>0.1±3</td>
<td>9.3±4.9</td>
<td>0.3±3</td>
<td>1.0±2</td>
<td>5.7±1.4</td>
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<td>EC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>22.5±2.4</td>
<td>19.8 (35)</td>
<td>462 (34)</td>
<td>579 (99)</td>
<td>515 (99)</td>
<td>4.2 (100)</td>
</tr>
</tbody>
</table>

3.2. In vitro assays

In vitro response data for these compounds at the cloned human 5-HT<sub>2A</sub> receptor subtypes is shown in Table 2. None of these compounds showed a high level of selectivity for any one of the individual receptor subtypes. Though R-DOI and 5-Ome DMT do have only a low 3- to 6-fold higher affinity for the 5-HT<sub>2A</sub> receptor relative to 5-HT<sub>2C</sub>, R-DOI does show a modest 28-fold higher affinity for the 5-HT<sub>2A</sub> receptor than the 5-HT<sub>2B</sub> receptor. AL-38022A and α-Me-5-HT show only a low 2- to 4-fold higher affinity for the 5-HT<sub>2C</sub> receptor than either the 5-HT<sub>2A</sub> or 5-HT<sub>2B</sub> receptors.

Table 3

Inhibition of radioligand binding to serotoninergic, adrenergic, and dopaminergic receptor subtypes and to transport systems by AL-38022A

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Radioligand</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; or Ki (nM)</th>
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</thead>
<tbody>
<tr>
<td>5-HT&lt;sub&gt;1A&lt;/sub&gt; (rat)</td>
<td>[3H]-8-OH-DPAT</td>
<td>294</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;1B&lt;/sub&gt; (rat)</td>
<td>[3H]-5-CT</td>
<td>5.570 (113±5)</td>
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<td>5-HT&lt;sub&gt;1D&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>6.900 (107±3)</td>
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<tr>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>1.2±0.6 (106±5)</td>
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<tr>
<td>5-HT&lt;sub&gt;2B&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>2.0±0.2 (104±2)</td>
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<tr>
<td>5-HT&lt;sub&gt;2C&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>0.87±0.2 (108±1)</td>
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<tr>
<td>5-HT&lt;sub&gt;2D&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>1.0±0.1 (100)</td>
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<tr>
<td>5-HT&lt;sub&gt;3A&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>9.6±3 (100)</td>
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<tr>
<td>5-HT&lt;sub&gt;3B&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>1.2±0.6 (106±5)</td>
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<tr>
<td>5-HT&lt;sub&gt;3C&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>3.0±0.3 (105±3)</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;4&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>1.9±0.8 (104±2)</td>
</tr>
<tr>
<td>5-HT&lt;sub&gt;5&lt;/sub&gt; (human)</td>
<td>[3H]-5-CT</td>
<td>0.87±0.2 (108±1)</td>
</tr>
<tr>
<td>Norepinephrine Transporter (r human)</td>
<td>[3H]-nisoxetine</td>
<td>100 000</td>
</tr>
</tbody>
</table>

*Performed at NovoScreen Biosciences, Corp. using their standardized screening protocols, r human denotes the recombinant human receptor. Inhibition constants (Ki) were determined using up to seven concentrations of each compound. Each value on the concentration plot was the mean of two determinations; IC<sub>50</sub> value, an average of at least two determinations performed at Alcon using cell or tissue preparations expressing cloned or native human receptors of interest.
inhibition at a non-selective sigma receptor at a concentration of 10 µM. However, this concentration is significantly greater than the nanomolar affinity and potency AL-38022A exhibits for the 5-HT₂ receptor subtypes.

3.3. Animal studies

3.3.1. Drug discrimination studies in rats

Nine animals were trained to discriminate intraperitoneal injection of DOM (1.0 mg/kg) from saline vehicle such that they consistently made >80% of their responses on the DOM-appropriate lever following administration of this drug dose. Administration of lower DOM doses (Fig. 2) resulted in the animals making fewer lever following administration of this drug dose. Administration of that engendered ≥1.45 mg/kg; 2.79–CL=0.03–R animals; 312–CL=0.2–R administered saline vehicle (13.1±2.1 responses/min).

Various doses of AL-38022A were examined in the DOM-trained animals; R-DOI and 5-O-Me DMT were examined for purpose of comparison (Fig. 2). AL-38022A produced a maximum of 99% DOM-appropriate responding (at 0.3 mg/kg), and stimulus substitution occurred in a dose-dependent manner (ED₅₀=0.05 mg/kg, 95% CL=0.03–0.10 mg/kg; 0.18 µmol/kg). Likewise, the DOM stimulus generalized to R-DOI (ED₅₀=0.2 mg/kg, 95% CL=0.1–0.3 mg/kg; 0.57 µmol/kg) and 5-O-Me DMT (ED₅₀=0.86 mg/kg, 95% CL=0.50–1.45 mg/kg; 2.79 µmol/kg). The animals’ response rates at drug doses that engendered ≥80% DOM-appropriate responding were not substantially different (p>0.05) from those observed following administration of saline vehicle (13.1±2.1 responses/min).

Figure 2. Percent drug-appropriate responding (±S.E.M.) following administration of AL-38022A, R-DOI and DOM to saline vehicle (saline=13.1±3.1 responses/min). Rate of responding is shown in the lower panel in responses per second.

3.3.2. Drug discrimination studies in monkeys

All monkeys reliably discriminated between saline and DOM. Saline occasioned less than 5% responding on the DOM-associated lever (upper panel, Fig. 3, filled symbols above “V”) whereas DOM dose-dependently increased responding on the drug-associated lever up to a maximum of 100% at the training dose of 0.32 mg/kg (upper panel, Fig. 3, open circles). AL-38022A also occasioned 80% DOM-appropriate responding at a dose of 0.1 mg/kg (upper panel, Fig. 3, open circles). The ED₅₀ values (95% confidence limits) for the discriminative stimulus effects of DOM, R-DOI, and AL-38022A were 0.14 mg/kg (0.08–0.21 mg/kg; 0.57 µmol/kg), 0.04 mg/kg (0.02, 0.06 mg/kg; 0.11 µmol/kg), and 0.04 mg/kg (0.01–0.06 mg/kg; 0.15 µmol/kg), respectively.

The discriminative stimulus effects of the training dose of DOM were dose-dependently antagonized by MDL 100907 with less than 1% responding on the DOM-associated lever obtained when the training dose of DOM was administered in combination with 0.032 mg/kg of MDL 100907 (upper panel, Fig. 4, closed circles). Similarly, the DOM-like discriminative stimulus effects of AL-38022A were dose-dependently antagonized by MDL 100907; the dose of AL-38022A that alone occasioned 100% DOM-lever responding (0.1 mg/kg), occasioning less than 1% DOM-lever responding when administered in combination with 0.032 mg/kg of MDL 100907 (upper panel, Fig. 4, open circles). Moreover, the response rate-decreasing effects of AL-38022A were dose-dependently reversed by MDL 100907.

Two-way ANOVA revealed a significant main effect of MDL 100907 dose ([F(5, 15)]=12.08, p<0.0001) but no significant main effect of treatment ([F(1, 3)]=2.83, p>0.1) nor any significant interaction between treatment and MDL 100907 dose ([F(5,15)]=0.87, p>0.1). Post-hoc analysis revealed that doses of MDL 100907 larger than 0.00032 mg/kg significantly decreased the discriminative stimulus.
The phenylethylamine DOM produces a discriminative stimulus in rats that is believed to be mediated by actions at 5-HT2 receptors (Glenon, 1996). Other phenylethylamines, such as R-DOI, and certain indolealkylamines, such as 5-Ome DMT, have been previously shown to substitute in DOM-trained rats, and there is a significant correlation between DOM-stimulus generalization potency and 5-HT2 receptor affinity for an extended series of agents (Fiorella et al., 1995; Glenon et al., 1982; Glenon, 1996). As such, it might be anticipated that agents that substitute in DOM-trained animals exert agonist actions at 5-HT2 receptors. Consistent with the high affinity and agonist properties of AL-38022A, substitution was observed in rats discriminating DOM. Furthermore, on a molar basis, AL-38022A was found to be seven times more potent than DOM and three times more potent than R-DOI—two rather selective 5-HT2 receptor agonists. In contrast to the phenylethylamines, certain indolealkylamines are 5-HT2 receptor agonists but display reduced selectivity for this population of receptors. AL-38022A is rather unique for an indolealkylamine analog in that it is quite selective for 5-HT2 receptors (Table 3); furthermore, AL-38022A was about 15 times more potent than 5-Ome DMT. Additionally, it is of interest to note that the potency of AL-38022A in DOM-trained rats is comparable to that previously reported for d-lysergic acid diethylamide (EC50 = 0.052 mg/kg) in this same assay (Glenon et al., 1983).

In view of the potent DOM-like response observed for AL-38022A in rats, it was of interest to further assess its CNS effects in a recently developed non-human primate drug discrimination assay (Li et al., 2008). The discriminative stimulus effects of DOM in rhesus monkeys were completely antagonized by MDL 100907, confirming a role for 5-HT2A receptors. This finding is consistent with a previous report that MDL 100907 potently blocks the discriminative stimulus effects of DOM in rats (Li et al., 2007). MDL 100907 is a highly selective 5-HT2A receptor antagonist, with greater than 100-fold selectivity for 5-HT2A (Ki = 0.85 nM) as compared to 5-HT2C (Ki = 88 nM) receptors in radioligand binding studies (Kehne et al., 1996). AL-38022A fully substituted for the discriminative stimulus effects of DOM in rhesus monkeys and the DOM-like effects of this molecule were antagonized by MDL 100907 providing strong evidence that AL-38022A induces the discriminative stimulus effects via the activation of 5-HT2A receptors in rhesus monkeys.

In summary, AL-38022A is a selective 5-HT2 receptor full agonist that fully generalized to the DOM discriminative stimulus in both rats and monkeys, being as potent or more potent than other well known compounds of the ergoline class (d-lysergic acid diethylamide), the phenylethylamine class (R-DOI), and the indolealkylamine class (5-Ome DMT). The pharmacological profile of AL-38022A suggests that it could be a useful tool in defining 5-HT2 receptor signaling and receptor characterization where 5-HT may function as a neurotransmitter.

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Appendix A. Supplementary data


References
