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Pharmacological properties and discriminative stimulus effects of a novel and selective 5-HT_2 receptor agonist AL-38022A [(*S*)-2-(8,9-dihydro-7*H*-pyrano[2,3-*g*]indazol-1-yl)-1-methylethylamine]

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ABSTRACT

AL-38022A is a novel synthetic serotonergic (5-HT) ligand that exhibited high affinity for each of the 5-HT_2 receptor subtypes ($K_i \leq 2.2$ 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 (EC_{50} range: 1.9–22.5 nM) and human (EC_{50} range: 0.5–2.2 nM) 5-HT_2 receptor subtypes including $[Ca^{2+}]_i$ mobilization and tissue contractions with apparently similar potencies and intrinsic activities and was a full agonist at all 5-HT_2 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-HT_2 receptor signaling and receptor characterization where 5-HT may function as a neurotransmitter.

1. Introduction

Activation of serotonin-2A (5-HT_{2A}) 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-HT_{2A} 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-HT_{2A} 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 β_2-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-HT_2 receptors, that is, potential activation of multiple signal transduction pathways via inositol phosphates/diacylglycerol formation, arachidonic acid release, 2-arachidonylglycerol 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-HT_2 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-HT_{2A} 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-HT_2 receptor, with no significant agonist activity at the 5-HT_{1A} receptor or any significant affinity for other receptors, unlike tryptamine analogs which have

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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-HT_2 agonist is desired. However, as noted above, DOI activation of 5-HT_2 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-HT_2 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-HT_2 receptor subtype selectivity.

During the course of our investigation into the role that 5-HT_2 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-HT_2 receptor agonists. This pursuit was of particular interest in view of renewed interest in the clinical relevance of 5-HT_{2A} 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-7*H*-pyrano[2,3-*g*]indazol-1-yl)-1-methylethylamine] (Fig. 1), which demonstrate it to be a selective 5-HT_2 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-HT_2 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-7*H*-pyrano[2,3-*g*]indazol-1-yl)-1-methylethylamine 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-aminopropane 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).

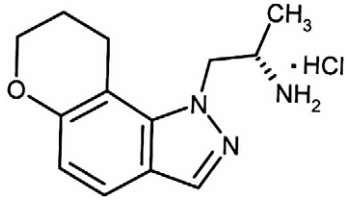

Fig. 1. Chemical structure of AL-38022A.

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 (C_1) of compound in buffer and the buffer concentration following extraction with 1-octanol (C_2) 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 $DC_{pH}=(C_1-C_2)/C_2$. Each determination was run in duplicate.

2.1.2. pK_a determination

The ionization constant was determined by potentiometric titration (Kyoto AT-310 Potentiometric Titrator) in water containing 10 to 40% acetonitrile. The nominal pK_a values obtained for each solvent mixture were plotted against the percentage of organic solvent to provide by extrapolation the pK_a 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 (T_{90}) 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-$HT_{2A/C}$ receptors

The procedure was previously described. (May et al., 2003a). In brief, the relative affinities of compounds at the 5-$HT_{2A/C}$ receptors were determined by measuring their ability to compete for the binding of the agonist radioligand [^{125}I]-DOI to rat brain 5-$HT_{2A/C}$ receptors. Aliquots of postmortem rat cerebral cortex homogenates (400 μl) dispersed in 50 mM Tris–HCl buffer (pH 7.4) were incubated with [^{125}I]-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 polypropylene 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 β-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-HT_2 receptors

Binding affinity of compounds at the cloned human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors expressed in Chinese hamster ovary cells using the agonist [^{125}I]-DOI (0.2 nM; 15 min at 37 °C) as the radioligand for each receptor was determined and reported as K_i

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 α_{2A} and α_{2C} adrenergic receptors

The procedure was previously described (May et al., 2006). Briefly, membranes from Sf9 cells expressing the cloned human α_{2A} or α_{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 [^{3}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 α_{2A} and α_{2C} and rat α_{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_3, 5-HT_{5A}, 5-HT_6 and porcine 5-HT_4 receptors, rat α_{1A} and α_{1B} adrenergic receptors, human D_1, D_{2L}, D_{2S}, $D_{4.2}$ 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 $[Ca^{2+}]_i$ mobilization assay

The procedure was previously described (Kelly et al., 2003; Sharif et al., 2006). Briefly, the receptor-mediated mobilization of intracellular calcium ($[Ca^{2+}]_i$) 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_{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 FT03 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 $[Ca^{2+}]_i$ mobilization assay

These assays were performed as for the 5-HT_{2A} receptor above, except that SR3T3 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 $[Ca^{2+}]_i$ mobilization assay

Functional response at the 5-HT_2 receptor subtypes was determined using CHO-K1 cells stably expressing mitochondrial-targeted bioluminescent aequorin, $G_{\alpha 16}$, 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_{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 β_2-adrenergic receptor activity

Agonist and antagonist functional activity was determined in immortalized human non-pigmented ciliary epithelial (NPE) cells that express endogenous β_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 β-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 produced 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

milk) using standard two-lever Coulbourn Instruments operant equipment as previously described (Glennon and Hauck, 1985; Young et al., 1980). Animal studies were conducted under an approved Institutional (VCU) Animal Care and Use Committee protocol.

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, The University of Texas Health Science Center at San Antonio, and with the 1996 Guide for the Care and Use of Laboratory Animals (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 ($DC_{7.4}$) 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 (5 ppm) it was estimated to have a $t_{1/2}$ of 15.1 years (25 °C), while at a higher concentration (1%) this value increased to >160 years. For comparison, *R*-DOI (5 ppm) was estimated to have a $t_{1/2}$ of >25 years; 5-HT and α-Me-5-HT have a $t_{1/2}$ of 11 days and 26 days, respectively, under similar experimental conditions (May et al., 2006).

3.2. In vitro assays

The *in vitro* binding and functional response data of AL-38022A, and for comparison, *R*-DOI, 5-OMe DMT, and 5-HT at the rat 5-HT_2 receptor subtypes are listed in Table 1. The affinity of *R*-DOI and AL-38022A at these receptors, K_i=0.1 nM and 0.13 nM, respectively, is comparable to that of 5-HT (0.3 nM), but approximately 10-fold higher than α-Me-5-HT and nearly 100-fold greater than 5-OMe DMT. Though the functional potency of *R*-DOI and AL-38022A is comparable (EC_{50}=19.5 nM and 22.5 nM, respectively) at the 5-HT_{2A} receptor, *R*-DOI has a significantly lower intrinsic activity (efficacy) (35% E_{max}, partial agonist) than AL-38022A, which has efficacy comparable to that of 5-HT and α-Me-5-HT (full agonist) at the endogenous rat 5-HT_{2A} receptor. 5-OMe DMT was a modestly potent partial agonist under these assay conditions [EC_{50}=462 nM (34% E_{max})]. Each of these compounds was a potent full agonist at the rat 5-HT_{2C} receptor with potency (EC_{50}) ranging from 1.8 nM for α-Me-5-HT to 77.8 nM for 5-OMe DMT.

In vitro response data for these compounds at the cloned human 5-HT_2 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_{2A} receptor relative to 5-HT_{2C}, *R*-DOI does show a modest 28-fold higher affinity for the 5-HT_{2A} receptor than the 5-HT_{2B} receptor. AL-38022A and α-Me-5-HT show only a low 2- to 4-fold higher affinity for the 5-HT_{2C} receptor than either the 5-HT_{2A} or 5-HT_{2B} receptors.

Table 1
In vitro binding and functional data at the endogenous rat 5-HT_2 receptors

	Rat 5-$HT^a_{2A/C}$	Rat 5-HT_2 EC_{50} nM (RI)		
	K_i nM	A[b]	B[c]	C[d]
AL-38022A	0.13±0.04	22.5±2.4 (87±1)	1.9±0.8	15.7±7.9 (89±9)
R-DOI	0.1[e]	19.8 (35)[f]	11.9[f]	30.2 (84)[f]
5-OMe DMT	9.3±4.9	462 (34)[g]	240[f]	77.8 (89)[f]
5-HT	0.3[e]	57.9 (99)[f]	3.5[f]	2.8 (94)[f]
α-Me-5-HT	1.0[e]	51.5 (99)[e]	4.2[f]	1.8 (100)[f]

[a]Rat cerebral cortex binding with [^{125}I]-DOI, value±SEM, K_d=44.1±12.3 pM (n=4) for [^{125}I]-DOI binding at the receptor; [b]Intracellular calcium mobilization in rat vascular smooth muscle cells (A7r5 cells) ±SEM, agonist response relative to 5-HT; [c]Rat stomach fundus contraction assays, value±SEM, n=3; [d]Intracellular calcium mobilization in murine SR3T3 cells expressing recombinant rat receptor, agonist response relative to 5-HT; [e]Data from (May et al., 2003a); [f]Data from (May et al., 2006); [g]Data from (May et al., 2003b) for completeness.

Table 2
In vitro cloned human receptor data

	5-HT_2 EC_{50} nM (RI)[a]			5-HT_2, K_i nM[b]		
	A	B	C	A	B	C
AL-38022A (n=4)	1.68±0.38 (113±5)	3.98±0.89 (81±2)	0.30±0.15 (107±3)	2.2±0.6	2.0±0.2	0.51±0.1
R-DOI[c] (n=3)	0.93±0.20 (106±1)	7.43±2.05 (84±3)	0.87±0.23 (108±1)	0.65	18	4.0
5-OMe DMT[c] (n=3)	3.40±1.00 (104±2)	23.1±7.30 (75±5)	9.60±2.92 (104±2)	15	52	42
5-HT[c] (n=2)	1.2±0.0 (108±6)	1.6±0.1 (113±6)	0.4±0.4 (100)	8.2	13	8.3
α-Me-5-HT[c] (n=3)	2.9±1.7 (100)	6.1±3.2 (100)	1.2±0.6 (106±0.6)	12	13	6.6

[a]Aequorin based $[Ca^{2+}]_i$ mobilization assay, values are the means of replicate experiments±SEM or range, agonist response relative to α-Me-5-HT for the 2A and 2B receptors and relative to 5-HT for the 2C receptor; [b]Cloned human receptors, [^{125}I]-DOI as radioligand, values are the mean of three determinations±SEM; [c]K_i data from (May et al., 2003b).

AL-38022A showed very low affinity (>1 μM) for other 5-HT receptors with the exception of 5-HT_{1A} where it showed modest affinity (K_i=294 nM), two orders of magnitude weaker than its affinity at the 5-HT_{2A} receptor (Table 3). Determination of the functional response of AL-38022A at the cloned human 5-HT_{1A} receptor showed it to have extremely low potency at this receptor [EC_{50}=15 μM (87%)]. Modest affinity was also observed for AL-38022A at the β_2-adrenergic receptor (K_i=480 nM); however, the functional response for this compound at the human β_2-adrenergic receptor was extremely low (EC_{50}>100 μM; IC_{50} against isoproterenol-induced cAMP production=17.4 μM). Only weak affinity (>1 μM) was observed for AL-38022A at α_1- and α_2-adrenergic receptors, the β_1-adrenergic receptor, dopamine receptors, and the norepinephrine and serotonin transporters (Table 3). Additionally, AL-38022A interacted only weakly with a number of other neurotransmitter-related receptors, ion channels, enzymes, and second messenger systems when tested at 1 nM, 100 nM, and 10 μM concentrations (Table S2, Supplementary data). The maximum inhibition of ligand binding induced by AL-38022A that was observed in any of these assays was 57% binding

Table 3
Inhibition of radioligand binding to serotonergic, adrenergic, and dopaminergic receptor subtypes and to transport systems by AL-38022A[a]

Receptor	Radioligand	IC_{50} or K_i (nM)
5-HT_{1A} (r human)	[^{3}H]-8-HO-DPAT	294
5-HT_{1B} (rat)	[^{125}I]-(−)-iodo-cyanpindol	5 570
5-HT_{1D} (human)	[^{3}H]-5-CT	6 900
5-HT_3 (human)	[^{3}H]-GR-65630	31 700[b]
5-HT_4 (pig)	[^{3}H]-GR-113808	30 300[b]
5-HT_{5A} (r human)	[^{3}H]-LSD	10 800[b]
5-HT_6 (r human)	[^{3}H]-LSD	19 600[b]
5-HT_7 (r human)	[^{3}H]-LSD	1 160
α_{1A} (rat)	[^{3}H]-7-MeO-prazosin	100 000[b]
α_{1B} (rat)	[^{3}H]-7-MeO-prazosin	74 000[b]
α_{2A} (r human)	[^{3}H]-MK-912	6 260
α_{2B} (r human)	[^{3}H]-MK-912	17 100
α_{2C} (r human)	[^{3}H]-MK-912	24 800
β_1 (human)	[^{3}H]-CGP-12177	5 000[b]
β_2 (human)	[^{3}H]-CGP-12177	480[b]
D_1 (human)	[^{3}H]-SCH-23390	138 000[b]
D_{2L} (human)	[^{3}H]-spiperone	>100 000[b]
D_{2S} (human)	[^{3}H]-spiperone	>100 000[b]
D_3 (rat)	[^{3}H]-spiperone	7 200[b]
$D_{4.2}$ (human)	[^{3}H]-spiperone	>100 000[b]
Norepinephrine Transporter (r human)	[^{3}H]-nisoxetine	>100 000
Serotonin Transporter (human)	[^{3}H]-*N*-Me-citalopram	62 500

[a]Performed at NovaScreen Biosciences, Corp. using their standardized screening protocols, r human denotes the recombinant human receptor. Inhibition constants (K_i) were determined using up to seven concentrations of each compound. Each value on the concentration plot was the mean of two determinations; [b]IC_{50} 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-HT_2 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 responses on the DOM-appropriate lever (ED_{50}=0.3 mg/kg, 95% CL=0.2–0.6 mg/kg; 1.25 μmol/kg). The animals' response rate following administration of different DOM doses (Fig. 2) was statistically similar ($p>0.05$) to their response rate following administration of saline vehicle (13.1±2.1 responses/min).

Various doses of AL-38022A were examined in the DOM-trained animals; *R*-DOI and 5-OMe 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 (ED_{50}=0.05 mg/kg, 95% CL=0.03–0.10 mg/kg; 0.18 μmol/kg). Likewise, the DOM stimulus generalized to *R*-DOI (ED_{50}=0.2 mg/kg, 95% CL=0.1–0.3 mg/kg; 0.57 μmol/kg) and 5-OMe DMT (ED_{50}=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 DOM or saline (Fig. 2).

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, filled circles). *R*-DOI occasioned responding on the DOM-associated lever up to a maximum of 100% at a dose of 0.1 mg/kg (upper panel, Fig. 3, open circles). AL-38022A also occasioned responding on the DOM-associated lever up to a maximum of 100% at a dose of 0.1 mg/kg (upper panel, Fig. 3, open squares). As compared to the saline (control) rate, DOM and *R*-DOI both dose-dependently decreased responding, with the average rate being 52% and 40% of the control rate at a dose of 0.32 DOM and 0.1 mg/kg *R*-DOI, respectively. AL-38022A, at the dose of 0.032 mg/kg, slightly increased responding (133% of the control rate) and at the dose (0.1 mg/kg) that fully substituted for DOM it markedly decreased responding (32% of the control rate; lower panel, Fig. 3, open squares). The ED_{50} 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.

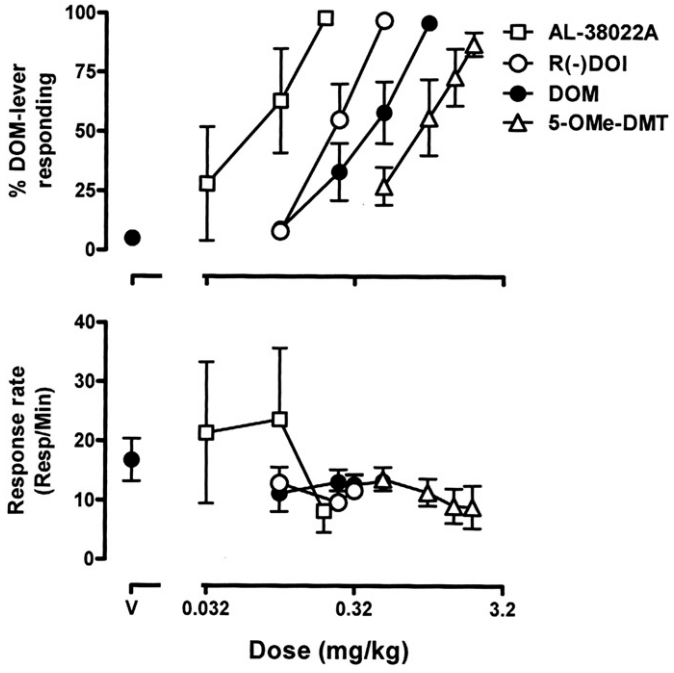

Fig. 2. Percent drug-appropriate responding (±S.E.M.) following administration of AL-38022A, *R*-DOI, DOM, and 5-OMe DMT to rats (n=6–9) trained to discriminate 1.0 mg/kg of DOM from saline vehicle (saline=5±2%) is shown in the upper panel. Response rate is shown in the lower panel (saline=13.1±3.1 responses/min).

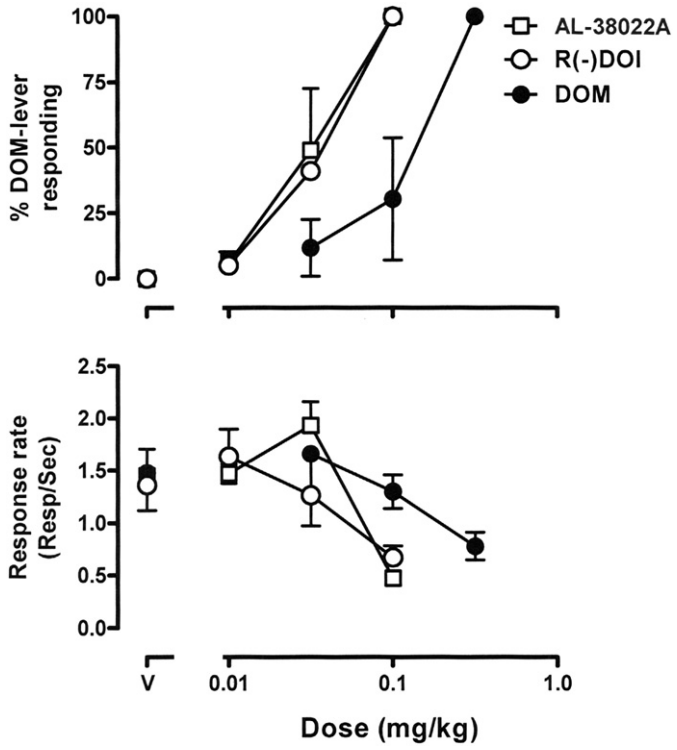

Fig. 3. Percent drug-appropriate responding (±S.E.M.) following administration of AL-38022A, *R*-DOI and DOM to rhesus monkeys (n=4) trained to discriminate 0.32 mg/kg of DOM from vehicle is shown in the upper panel. Rate of responding is shown in the lower panel in responses per second.

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 squares). 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

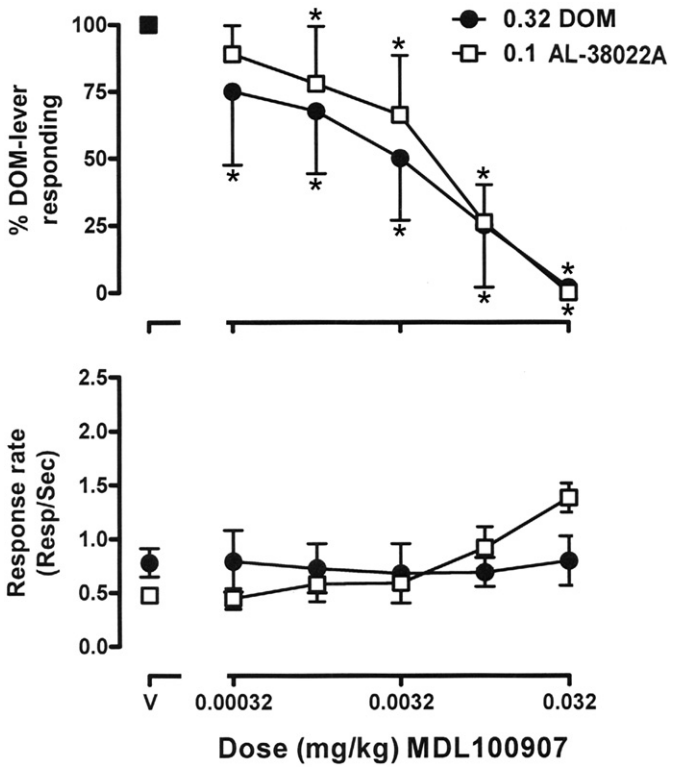

Fig. 4. Percent drug-appropriate responding (±S.E.M.) following administration of different doses of MDL 100907 in combination with 0.1 mg/kg AL-38022A or 0.32 mg/kg DOM to rhesus monkeys (n=4). See Fig. 3 for other details. * $p<0.05$ compared to either 0.32 mg/kg DOM or 0.1 mg/kg AL-38022A administered alone.

effects of 0.32 mg/kg of DOM while doses of MDL 100907 larger than 0.001 mg/kg significantly decreased the discriminative stimulus effects of 0.1 mg/kg of AL-38022A ($p<0.05$).

4. Discussion

The current studies have demonstrated that AL-38022A exhibits high affinity for 5-HT_2 receptor subtypes, but a significantly lower (>100-fold less) affinity for other 5-HT receptor subtypes, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{3-7} receptors. Furthermore, AL-38022A demonstrated only a very low affinity for a broad array of other physiologically relevant receptors (e.g., dopaminergic and adrenergic, etc.), ion channels, second messenger elements and neurotransmitter transport sites. AL-38022A potently stimulated functional responses via rat and human 5-HT_2 receptor subtypes (recombinant and native) including $[Ca^{2+}]_i$ mobilization and tissue contractions with apparently similar potencies and intrinsic activities and exhibited full agonist properties at all 5-HT_2 receptor subtypes. This overall profile is similar to that of *R*-DOI, but in contrast, AL-38022A shows full agonist response at 5-HT_2 receptors. Further, this selectivity profile is in sharp contrast to the pharmacological profile of *d*-lysergic acid diethylamide, which mediates its effects via multiple families of receptors including serotonergic, dopaminergic, and adrenergic (reviewed, Nichols, 2004). Therefore, AL-38022A represents a much more specific pharmacological tool with which to investigate the role of 5-HT_2 receptors in CNS-mediated functions.

AL-38022A is a compound with high solubility and excellent chemical stability in aqueous solutions. Its distribution coefficient is comparable to that of *R*-DOI and 5-OMe DMT, and the acidic dissociation constant (pK_a value) for AL-38022A (8.45) lies between that of *R*-DOI and *d*-lysergic acid diethylamide, 9.89 and 6.37 (Stoll et al., 1954), respectively. Based on its physicochemical properties, AL-38022A would not be anticipated to have a dramatically different in vivo distribution profile than that of the compounds mentioned above.

The phenylethylamine DOM produces a discriminative stimulus in rats that is believed to be mediated by actions at 5-HT_2 receptors (Glennon, 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-HT_2 receptor affinity for an extended series of agents (Fiorella et al., 1995; Glennon et al., 1982; Glennon, 1996). As such, it might be anticipated that agents that substitute in DOM-trained animals exert agonist actions at 5-HT_2 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-HT_2 receptor agonists. In contrast to the phenylethylamines, certain indolealkylamines are 5-HT_2 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-HT_2 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 (EC_{50}=0.052 mg/kg) in this same assay (Glennon 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-HT_{2A} 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-HT_{2A} receptor antagonist, with greater than 100-fold selectivity for 5-HT_{2A} (K_i=0.85 nM) as compared to 5-HT_{2C} (K_i=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-HT_{2A} receptors in rhesus monkeys.

In summary, AL-38022A is a selective 5-HT_2 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-HT_2 receptor signaling and receptor characterization where 5-HT may function as a neurotransmitter.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbb.2008.07.015.

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