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The discovery of 071031B, a novel serotonin and noradrenaline reuptake inhibitor

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HIGHLIGHTS

- We establish a system to screen monoamine reuptake inhibitors fast and accurately.
- Using this system, we discover a potential antidepressant 071031B.
- 071031B has robust antidepressant effect in vivo and transporter affinity in vitro.
- 071031B is expected to be a novel serotonin and noradrenaline reuptake inhibitor.

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ABSTRACT

Depression is a severe mood disorder with increasing morbidity and suicidality, while the current therapy is not satisfactory. Serotonin and noradrenaline reuptake inhibitors (SNRIs) have been reported to have higher efficacy and/or faster acting rate than commonly used antidepressants. The present study was designed to screen the potential SNRIs, using in vitro radioligand receptor binding assays and in vivo animal tests, and introduced the discovery of 071031B. In the tail suspension test and forced swimming test in mice, six compounds (071017S, 071026W, 071031A, 071031B, 080307A and 080307B) showed robust antidepressant activity, without stimulant effect on the locomotor activity or other side effects, and the minimal effective dose of 071017S, 071026W, 071031A and 071031B was less than that of duloxetine; in vitro binding tests indicated that 071031B had high affinity to both serotonin transporter and noradrenaline transporter with similar inhibitory rates to duloxetine at 1 and 100 nM; acute toxicity test indicated that the LD_{50} value of 071031B was similar to that of duloxetine. These findings demonstrated that this integrated system, combining high throughput screening technology and in vivo animal tests, is effective to screen potential monoamine reuptake inhibitors fast and accurately; 071031B is expected to be a novel serotonin and noradrenaline reuptake inhibitor for its robust antidepressant activity and transporter affinity.

1. Introduction

Depression is the most common mood disorder, with increasing mortality, suicidality, and heavy burden to society [8]. Currently, pharmacotherapy is the most effective strategy to treat depression, and most available antidepressants exert therapeutic activity by activating monoaminergic neural transmission. According to their characteristics and mechanism of action, commercially available antidepressants are classified as: (1) monoamine reuptake inhibitors, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (NRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and norepinephrine and dopamine reuptake inhibitors (NDRIs); (2) monoamine oxidase inhibitors (MAOIs), including irreversible nonselective MAOIs and reversible selective inhibitor of monoamine oxidase-A (e.g. moclobemide); (3) other antidepressants with multiple targets, e.g. Mirtazapine [10], with α_2-adrenoceptor/5-HT_2/5-HT_3 receptor antagonistic activity; vilazodone [6], with selective serotonin reuptake and 5-HT_{1A} receptor partial agonistic activity; agomelatine [7], with melatonergic agonistic (at

Abbreviations: TCAs, tricyclic antidepressants; SSRIs, selective serotonin reuptake inhibitors; NRIs, norepinephrine reuptake inhibitors; SNRIs, serotonin and norepinephrine reuptake inhibitors; NDRIs, norepinephrine and dopamine reuptake inhibitors; MAOI's, monoamine oxidase inhibitors; 5-HT, serotonin; R&D, Research & Development; SERTs, serotonin transporters; NETs, norepinephrine transporters; TST, tail suspension test; FST, forced swimming test; LD_{50}, median lethal dosage; ANOVA, one-way analysis of variance; CNS, central nervous system.

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both MT_1 and MT_2 receptors) and 5-HT_{2C} antagonistic properties.

As we look back 60 years to the discovery of the first antidepressant, Research & Development (R&D) strategies for novel antidepressants experienced several phases: from by chance discovery strategy, to single target discovery strategy, then to multitarget discovery strategy. In 1950s, TCAs and nonselective MAOIs, the first generation of antidepressants, were discovered absolutely by chance [5,9]. However, besides therapeutic targets, i.e. serotonin transporter (SERT) and noradrenaline transporter (NET), TCAs also had high affinity for adrenergic, muscarinic, and other receptors, which led to severe side effects. Then, most drug discovery projects aimed to discover an antidepressant that was highly selective for a single target, and SERT was chosen as the most commonly studied target due to the close relationship between serotonin and depression. We must admit that fluoxetine, the first SSRI to be marketed, represent an important milestone in the history of antidepressants [20]. However, the efficacy and onset of SSRIs were still not satisfactory. How to accelerate onset of action and increase response rate has been a crucial point for antidepressant R&D. As is well-known, complex diseases, such as depression, are not caused by a single gene, transmitter, or system. Accordingly, by modulating multiple targets simultaneously, drugs may have the potential to provide superior efficacy. SNRIs, activating serotonergic and noradrenergic neurotransmission simultaneously, have been reported to have higher efficacy and/or faster acting rate than commonly used antidepressants [23]. SNRIs have been first-line antidepressants, and the economic revenue is attractive to pharmaceutical companies. Taking duloxetine as the leading compound, we synthesized lots of compounds with novel structures. The present study was designed to screen potential SNRIs using the integrated evaluation system established by our group [24], which combined in vitro high throughput screening technology, and in vivo animal tests, and find novel SNRI candidate with high efficacy and independent intellectual property. This study, by in vivo and in vitro screening and comparison, led to the discovery of 071031B, a novel serotonin and noradrenaline reuptake inhibitor.

2. Materials and methods

2.1. Animals

Male or female ICR mice weighing 18–20 g and male SD rats weighing 180–200 g were purchased from Beijing Vital River Laboratory Animal Technology Company (Beijing, China). Animals were group housed in rooms maintained at $22 \pm 2\,^{\circ}C$, with humidity of 40–60% and 12 h:12 h-light/dark cycle (lights on at 8:00 am). Experiments were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1996).

2.2. Drugs and reagents

Fourteen compounds with novel structure were synthesized in Beijing Institute of Pharmacology and Toxicology (Beijing, China). Duloxetine hydrochloride was purchased from Beijing Furenkang biopharmaceutical corporation, Ltd (Beijing, China). [^{3}H]citalopram and [^{3}H]nisoxetine were purchased from PerkinElmer Life Sciences (NEN, Boston, MA, USA). Fluoxetine and desipramine were purchased from Sigma (St. Louis, MO, USA).

2.3. Tail suspension test in mice

The tail suspension test (TST) was performed according to that described previously [18,25]. Mice were treated with various doses of novel compounds (intraperitoneal injection, i.p.) 0.5 h before TST; then, mice were suspended 5 cm above the bottom of apparatus. The duration of immobility during the last 4 min of the total 6 min was recorded. Mice were judged to be immobile when they hung passively without moving. Inhibition rate was calculated by the following formula: Inhibition rate (%) = (immobility time of vehicle group − immobility time of compound group) × 100/immobility time of vehicle group.

2.4. Forced swimming test in mice

The forced swimming test (FST) in mice was performed according to that described previously [13,25]. Thirty minutes after intraperitoneal injection of various doses of novel compounds (2.5–20 or 5–30 mg/kg), mice were individually placed in glass cylinders (diameter 10 cm, height 20 cm) containing 10 cm of water maintained at 25 °C. The duration of immobility during the last 4 min of the total 6 min was measured. Mice were considered to be immobile when they floated motionless, making only the movement necessary to keep their heads above the water. Inhibition rate was calculated by the same formula as mentioned above.

2.5. Locomotor activity test in mice

Compounds were injected intraperitoneally 30 min prior to tests, then mice were placed in the corner of a plastic box and allowed to habituate for 5 min. As for 071031A, locomotor activity was determined using VIDEOMEX-V image analytic system (Columbus Instruments, USA), and traveling distance in 5 min was recorded. As for 071017S, 071026W, and 071031B, locomotor activity was determined using visual method, and parameters including the number of crossing and number of rearing were recorded in 5 min.

2.6. Binding affinity for rat SERTs or NETs

The affinity of compounds for rat SERTs or NETs was determined by competition of [^{3}H]citalopram or [^{3}H]nisoxetine, according to that described previously [1,21]. Membrane protein was prepared from rat frontal cortex. Competitive binding assays were performed in reaction buffer containing 20 μg membrane protein, 1.25 nM [^{3}H]citalopram or 1.0 nM [^{3}H]nisoxetine, and various concentration of compounds (1 and 100 nM) at 25 °C (SERTs) or 4 °C for 60 min (NETs). Nonspecific binding was determined using10 μM fluoxetine or 10 μM desipramine. Inhibition rate was calculated by the following formula: Inhibition rate = (total binding − drug binding) × 100%/(total binding − nonspecific binding). All data are presented as mean values of three dependent assays.

2.7. Acute toxicity test

Acute toxicity test was conducted with 071031B in mice using intraperitoneal administration. Male or female mice were treated with various doses of 071031B (98.0, 89.1, 81.0, or 73.6 mg/kg), and observed for 2 weeks. Toxic symptoms and mortality rates were recorded, and the median lethal dosage (LD_{50}) and 95% confidence interval values were calculated.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Prism 5.0, version 2.0; GraphPad Software Inc., San Diego, CA). Data from the TST, FST, and locomotor activity test were showed as means ± SD, and were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. The transporter binding data were analyzed using one-site nonlinear regression of concentration–effect curve. The LD_{50} and 95% confidence interval