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Novel Chiral 1,3,4-Oxadiazole Derivatives Inducing Astrocyte Differentiation of Rat Fetal Neural Stem Cells

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Neural stem cells (NSCs) are multipotent cells that can differentiate into neurons, astrocytes, or oligodendrocytes. The primary focus of research on NSCs has been their neuronal differentiation to treat neurodegenerative diseases including Alzheimer's disease and Parkinson's disease.[1,2] Many efforts have been made to drive NSCs toward a neuronal fate, not only using transcription factors but also small molecules.[3–5] However, there have been only a few studies reporting the differentiation of NSCs into astrocytes. Astrocytes are the most abundant cell type in the brain.[6] Astrocytes are found to be multifunctional cells that play critical roles in the development of neurons, maintenance of cellular homeostasis, and energy supply.[5] Furthermore, they form in the brain together with pericytes, endothelial cells, and adjacent neurons the blood–brain barrier.[5] As the knowledge about the role of astrocytes expanded, the importance of astrocytes in neurodegenerative diseases started to emerge. Thus, neuroscience research might benefit from small molecules that regulate the fate of NSCs.

We reported that 1,3,4-oxadiazole derivatives induce the differentiation of rat fetal neural stem cells into astrocytes.[7] The previously reported compounds were racemic mixtures. The stereochemistry of small molecules is very important for their biological activity. The enantiomers of many chiral drugs have been reported to show different biological activities.[8] Thus, we tried to explore the activity of the stereoisomers of 1,3,4-oxadiazole derivatives.

Therefore, we synthesized the derivatives of compound **1** (Figure 1). A set of *S*- and *R*-enantiomers was prepared to determine their stereospecific activity on NSC differentiation. The desired compounds were readily synthesized from *N*-Boc phenylglycines, a commercially available chiral building block, as starting materials using the same methods as described previously (Scheme 1).[7]

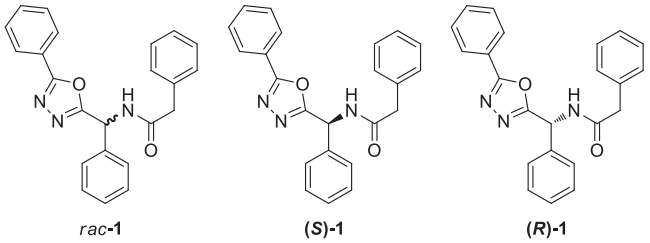
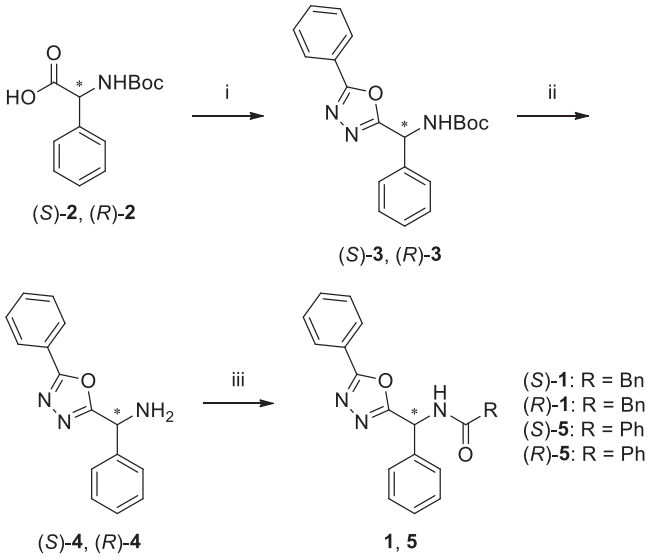


Figure 1. Structure of compound *rac*-**1** and its stereoisomers.

An image-based assay was performed as reported previously,[7] which revealed that the (*S*)-isomer of compound **1** induces the differentiation of rat fetal neural stem cells into astrocytes (data not shown). The racemate of benzamide compounds **5** derived from **1** exhibited an increased activity for astrocyte differentiation. The (*S*)-isomer (*S*)-5[9] of the benzamide analogue showed a significantly increased activity for astrocyte differentiation compared to the (*R*)-isomer (*R*)-5 (Figure 2).

An additional evaluation of compounds (*S*)-1, (*S*)-5, and (*R*)-5 was conducted using real-time (RT)-PCRs of markers for astroglia (*GFAP* and *S100*), neurons (*β-tubulin*), and



Scheme 1. Synthesis of **1** and **5**. Reagents and conditions: (i) benzhydrazide, CDI, CBr_4, PPh_3, DCM, 0 °C, 66–78%; (ii) TFA, DCM, rt., 2 h, 99%; (iii) compound (*S*)-**1**: Phenylacetyl chloride, THF, rt., 5 h, 99%, compound **5**: Benzoic acid, DMAP, EDC, DCM, rt., 4 h, 26–48%.

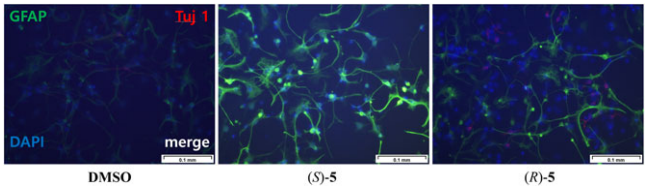


Figure 2. Representative immunofluorescence images of GFAP (astrocyte marker, green), DAPI (nuclei, blue), and β-tubulin(neural marker, red) of NSCs treated with DMSO, (*S*)-5, or (*R*)-5.

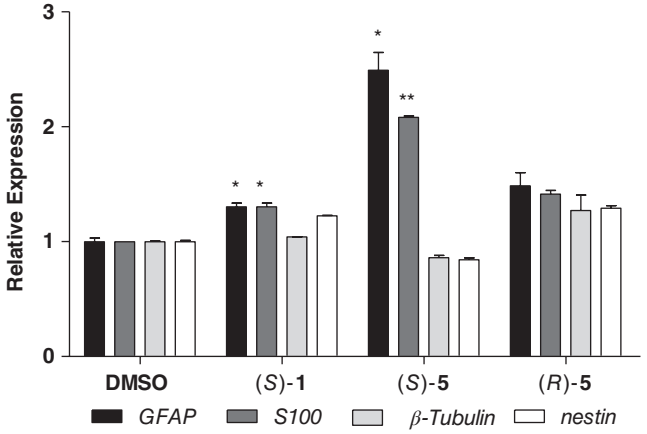


Figure 3. Relative mRNA expression levels of *GFAP*, *S100*, *β-tubulin*, and *nestin* after the treatment of astrocytes with (*S*)-**1**, (*S*)-**5**, and (*R*)-**5**, which were analyzed using quantitative real-time PCR. Data present mean ± SEM. Student *t* test, $^{*}P < 0.05$, $^{**}P < 0.01$.

neural stem cells, progenitors, or neurons (*nestin*). Treatment of NSCs with (*S*)-5 significantly increased the expression of both astroglial markers more than 2-fold compared to DMSO used as vehicle control, while (*S*)-1 and (*R*)-5 induced only 1.2- and 1.3-fold increases, respectively (Figure 3). Furthermore, (*S*)-5 did not enhance (or slightly decreased) the expression of *β-tubulin* and *nestin* (Figure 3), while (*R*)-5 slightly and significantly increased the expression of these neuronal markers.

In summary, we identified (*S*)-5 as a potent small molecule regulator for the selective differentiation of NSCs into astrocytes. The activity of (*S*)-5 for the differentiation of NSCs into astrocytes was validated by image-based assay and RT-PCR. Only the (*S*)-isomer (*S*)-5 potently increased the expression of astroglial markers while the corresponding (*R*)-isomer did not significantly influence neuronal and astroglial markers. This study demonstrates that the chirality of (*S*)-5 was an important factor for its activity in astrocytogenesis and could be a chemical tool for stem cell researches.

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9. ^{1}H and ^{13}C NMR and HRMS data of compound (*S*)-**5**: (*S*)-*N*-(phenyl(5-phenyl-1,3,4-oxadiazol-2-yl)methyl)benzamide ((*S*)-5). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.74 (d, *J* = 7.8 Hz, 1H), 7.97 (t, *J* = 6.8 Hz, 4H), 7.66–7.54 (m, 6H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.40 (d, *J* = 7.2 Hz, 1H), 6.73 (d, *J* = 7.8 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.20, 165.83, 164.47, 136.81, 133.41, 132.13, 131.77, 129.49, 128.62, 128.36, 128.29, 127.92, 127.71, 126.54, 123.19, 49.53; HRMS (ESI): *m/z* calcd for $C_{22}H_{18}N_3O_2$ $[M+H]^+$ 356.1394, found 356.1410.