Article

Copper-Catalyzed Synthesis, Bio-Evaluation, and in Silico Studies of 2-Aryl-N-Alkylbenzimidazoles as Neuroprotective Agents

Yun-Xin Yao 1,2, Nan-Nan Jia 1, Ya-Nan Cao 1, Xing-Xiu Chen 3, Feng Gao 1,2,* and Xiao-Xia Liang 1,*

1 Agronomy College, Sichuan Agriculture University, Chengdu 611130, China; yaoyunxin@my.swjtu.edu.cn (Y.-X.Y.); jnan168@126.com (N.-N.J.); caoyanan2016@163.com (Y.-N.C.)
2 School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China
3 Department of Chemistry and Biochemistry, University of Oklahoma, 101 Stephenson Parkway SLSRC, Norman, OK 73019-5251, USA; chenxingxiu@163.com
* Correspondence: gaof@swjtu.edu.cn (F.G.); liangxiaoxia@sicau.edu.cn (X.-X.L.);
Tel.: +86-132-5836-2006 (F.G.); +86-181-8199-6903 (X.-X.L.)

Received: 8 September 2018; Accepted: 28 September 2018; Published: 30 September 2018

Abstract: 2-aryl-N-alkylbenzimidazole derivatives synthesized by CuI/PPh₃ promoted direct coupling of N-alkylbenzimidazoles with aryl bromides. In vitro neurotoxicities of 20 compounds were evaluated, and the neuroprotective abilities of low-neurotoxic compounds (3b, 3g, 3h, 3i, 3j, 3k, 3o, 3q, 3s and 3t) were investigated against toxicity induced by 1-methyl-4-phenylpyridinium ion (MPP⁺) in SH-SY5Y neuronal cells. In silico studies revealed that compound 3g could have molecule docking with the following proteins: the bone morphogenetic protein receptor type 1B (BMPR1B), human cytochrome P450 1B1 (CYP1B1), Metabotropic glutamate receptor 7 (GRM7), histone deacetylase 6 (HDAC6), 5-hydroxytryptamine receptor 5A (HTR5A), human topoisomerase II beta (TOP2B). A molecular docking simulation of model compound 3g and model protein CYP1B1 has been shown.

Keywords: 2-arylbenzimidazoles; arylation; copper-catalysis; neuroprotective effect; molecular docking

1. Introduction

Benzimidazole derivatives are known to display a wide range of biological activities [1]. Among them, the neuroprotective activity is significant in the treatment of neurodegenerative diseases including ischemia [2], epilepsy [3], and Alzheimer’s and Parkinson’s disease [4]. For example, two clinically-promising anti-Parkinson’s disease agents, Istradefylline and Preladenant, are both benzimidazole derivatives [5]. In particular, 2-arylbenzimidazole derivatives were evaluated as inhibitors of c-Jun N-terminal kinase 3 (JNK3) in the treatment of neurodegenerative symptoms [6,7]. In addition, a novel cholinesterase (ChE) inhibitor with 2-arylbenzoimidazoles skeleton was helpful in reversing the memory impairments associated with Alzheimer’s disease [8]. 2-arylbenzimidazole derivatives also inhibited the increase of inducible nitric oxide synthase (iNOS) when peripheral nerve injury occurred (Figure 1) [9]. Consequently, there is a great demand for the generation a library of novel 2-arylbenzimidazoles for the evaluation of their neuroprotective activity.

The preparations of 2-arylbenzimidazoles include intermolecular cyclization of aldehydes [10–12], intramolecular cyclization of alobenzanilides, and oxidative arylation of enzothiazoles by recyclization [13–16]. Another approach to synthesizing 2-arylbenzimidazoles is metal-catalyzed direct C–H bond arylation reactions, which have emerged as one of the most efficient protocols for the synthesis of diverse 2-arylbenzimidazoles. The classical cross-coupling of benzimidazoles with aryl electrophiles catalyzed by a noble metal such as Palladium has yielded various 2-arylbenzimidazoles [17–22]. However,
the high cost limits the scale-up development of Pd-catalyzed cross coupling reaction. Fortunately, cheap copper is an efficient counterpart of palladium in the direct arylation of benzoheterocyclic compounds [23–28]. Previously, we have reported that Cu/PPh₃ directly catalyzed the 2-arylation of benzoxazoles with economical aryl bromides [29], and the same catalysis system has been used in our further research. In this work, a series of 2-aryl-N-alkylbenzimidazole derivatives were prepared via copper-catalyzed direct arylation of N-alkylbenzimidazoles with aryl bromides, and their in vitro neuroprotective activities were evaluated.

2. Results and Discussion

2.1. Copper-Catalyzed Synthesis of 2-Aryl-N-Alkylbenzimidazoles

We investigated the best reaction conditions of the Cu-catalyzed direct arylation of 1-methyl-benzimidazole (1a) with 1-bromo-4-tert-butylbenzene (2d) (Table 1). Initially, the screening of various bases was carried out under the CuI/PPh₃ (10 mol% /30 mol%) catalytic system at 140 °C in DMF for 24 h. There was small quantity of 2-[4-(1,1-Dimethylethyl)phenyl]-1-methyl-1H-benzimidazole (3d) when KOH, NaOH and i-BuOK (Table 1, entry 1–3) was used as base. In contrast, a mild base such as K₂CO₃, Na₂CO₃ or KH₂PO₄ (Table 1, entry 4–6) promoted the arylation reaction dramatically. Next, we examined the effect of temperatures ranging from 120 °C to 150 °C (Table 1, entries 4, 7–8). When the temperature decreased to 120 °C, the yield dropped to 45%. After the temperature screening, the selection of a high boiling-point solvent drew our attention. DMF and xylene positively affected this arylation reaction. We also studied the reasons why, as the temperature changed from 140 °C to 130 °C (Table 1, entries 9 and 10), the solvent became xylene. The yield increased as the temperature rose to 140 °C. The reaction-time difference of group 1 (Table 1, entries 11 and 8) and group 2 (Table 1, entries 9 and 12) revealed that increasing time benefits the yield. Therefore, optimized conditions (Table 1, entry 12) were obtained by following the scope of aryl bromides. In a study of 2-arylations of 1-methyl-benzimidazole (Table 2), compared with optimized conditions using 40 h, the majority of products could be produced in the same yield with a shorter reaction time (24 h).

Table 1. Optimization of direct 2-arylation of 1-methyl-benzimidazole 1a with 1-bromo-4-tert-butylbenzene 2d.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KOH</td>
<td>DMF</td>
<td>140</td>
<td>24</td>
<td>Trace</td>
</tr>
<tr>
<td>2</td>
<td>NaOH</td>
<td>DMF</td>
<td>140</td>
<td>24</td>
<td>Trace</td>
</tr>
<tr>
<td>3</td>
<td>i-BuOK</td>
<td>DMF</td>
<td>140</td>
<td>24</td>
<td>Trace</td>
</tr>
<tr>
<td>4</td>
<td>K₂CO₃</td>
<td>DMF</td>
<td>140</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>Na₂CO₃</td>
<td>DMF</td>
<td>140</td>
<td>24</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>KH₂PO₄</td>
<td>DMF</td>
<td>140</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>K₂CO₃</td>
<td>DMF</td>
<td>120</td>
<td>24</td>
<td>45</td>
</tr>
<tr>
<td>8</td>
<td>K₂CO₃</td>
<td>DMF</td>
<td>150</td>
<td>24</td>
<td>64</td>
</tr>
<tr>
<td>9</td>
<td>K₂CO₃</td>
<td>Xylene</td>
<td>130</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>K₂CO₃</td>
<td>Xylene</td>
<td>140</td>
<td>24</td>
<td>75</td>
</tr>
<tr>
<td>11</td>
<td>K₂CO₃</td>
<td>DMF</td>
<td>150</td>
<td>40</td>
<td>69</td>
</tr>
<tr>
<td>12</td>
<td>K₂CO₃</td>
<td>Xylene</td>
<td>150</td>
<td>40</td>
<td>78</td>
</tr>
</tbody>
</table>

* The reactions performed using 1.0 equiv. of 1a, 1.2 equiv. of 2d and 2.0 equiv. of base on a 0.1 mmol scale. b This is NMR assay yield. c Isolated yield.

The use of the aryl bromides in the 2-arylation of 1-methyl-benzoimidazole is presented in Table 2. Under optimized reaction conditions, the yields of aryl bromides with electron neutral groups, 2-bromonaphthalene (2a), 1-bromonaphthalene (2e), and 1-bromo-4-tert-butylbenzene (2d), were 72%,
85%, and 78% respectively. The aryl bromides bearing electron-donating methoxy (2g) groups and electron-withdrawing fluoric (2c) and trifluoromethyl (2f) groups were well tolerated in moderate yields. In addition, the direct arylation of 1-methyl-benzimidazole with heteroaryl bromides including 3-bromoquinoline (2b), 2-bromothiophene (2h), and 2-bromopyridine (2i) proceeded well to produce yields of 80%, 70%, and 54%, respectively.

Table 2. Scope of aryl bromides in direct 2-arylation of 1-methyl-benzimidazole 1a.

<table>
<thead>
<tr>
<th>Aryl Bromide</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a (72%)</td>
<td></td>
</tr>
<tr>
<td>3b (80%)</td>
<td></td>
</tr>
<tr>
<td>3c (69%)</td>
<td></td>
</tr>
<tr>
<td>3d (78%)</td>
<td></td>
</tr>
<tr>
<td>3e (85%)</td>
<td></td>
</tr>
<tr>
<td>3f (70%)</td>
<td></td>
</tr>
<tr>
<td>3g (72%)</td>
<td></td>
</tr>
<tr>
<td>3h (70%)</td>
<td></td>
</tr>
<tr>
<td>3i (54%)</td>
<td></td>
</tr>
</tbody>
</table>

With respect to 2-arylation of 1-ethyl-benzimidazole (Table 3), the scope could be extended when it reacted with 1-bromo-4-fluorobenzene (2c), 2-bromothiophene (2h), 2-bromonaphthalene (2a) and 3-bromoquinoline (2b), which delivered the arylated products (3j, 3k, 3n and 3m) in 40–46% yields. 4-Bromobenzotrifluoride (3l) was less tolerated in 20%. The arylation scope of 1-benzyl-benzimidazole was also shown in Table 3. In most cases, 1-benzyl-benzimidazoles (3o, 3p, 3q, 3s and 3t) were arylated at yield rates 40–54%. The challenging product, 1-(phenylmethyl)-2-(2-pyridinyl)-1H-benzimidazole (3r), was produced at a yield rate of 35%. Although yields of heterocyclic structures (Table 3. 3k and 3m, Table 4. 3r, 3s and 3t) were moderate, the economical Cu-catalyzed protocol helped to meet the demands of the activity tests.
Table 3. Scope of aryl bromides in direct 2-arylation of 1-ethyl-benzoimidazole 1b and 1-benzylbenzimidazole 1c.

<table>
<thead>
<tr>
<th>Aryl Bromide</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3j</td>
<td>42%a</td>
</tr>
<tr>
<td>3k</td>
<td>40%a</td>
</tr>
<tr>
<td>3l</td>
<td>20%b</td>
</tr>
<tr>
<td>3m</td>
<td>46%a</td>
</tr>
<tr>
<td>3n</td>
<td>40%a</td>
</tr>
<tr>
<td>3o</td>
<td>50%</td>
</tr>
<tr>
<td>3p</td>
<td>43%c</td>
</tr>
<tr>
<td>3q</td>
<td>54%</td>
</tr>
<tr>
<td>3r</td>
<td>35%d</td>
</tr>
<tr>
<td>3s</td>
<td>53%</td>
</tr>
<tr>
<td>3t</td>
<td>50%</td>
</tr>
</tbody>
</table>

a 1b was recovered from 50% to 60% yield. b 1b was recovered in 78% yield. c 1c was recovered from 55% yield. d 1c was recovered in 62% yield.

2.2. Neuroprotective Performances of 2-Aryl-N-Alkylbenzimidazoles against MPP⁺

The ability to prevent SH-SY5Y cells from MPP⁺-induced damage is shown in Table 4 and Figure 1. Unfortunately, our results indicated that the protective capabilities of compounds (3b, 3g, 3h, 3i, 3j, 3k,
3o, 3q, 3s, 3t) under 10 and 50 μM were low. We chose these compounds for the aforementioned experiment based on the fact that they did not show appreciable neurotoxicities on SH-SY5Y cells under 50 μM. (The inhibition rates of 20 compounds are illustrated in the supplementary materials).

<table>
<thead>
<tr>
<th>Conc. (μM)</th>
<th>3b</th>
<th>3g</th>
<th>3h</th>
<th>3i</th>
<th>3j</th>
<th>3k</th>
<th>3o</th>
<th>3q</th>
<th>3s</th>
<th>3t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protective rate (%)</td>
<td>50</td>
<td>−42.99</td>
<td>−10.2</td>
<td>0.79</td>
<td>2.23</td>
<td>−2.23</td>
<td>1.97</td>
<td>−17.82</td>
<td>−2.72</td>
<td>2.04</td>
</tr>
<tr>
<td>10</td>
<td>−4.72</td>
<td>16.19</td>
<td>0.13</td>
<td>0.92</td>
<td>−4.19</td>
<td>5.64</td>
<td>0.41</td>
<td>0.44</td>
<td>2.99</td>
<td>3.13</td>
</tr>
</tbody>
</table>

Figure 1. Protective rate (%) under MPP⁺ treatment.

2.3. The Computer Analysis of Neuroprotective Targets Metabolising 3g and Docking Analysis

In order to gain some insights into the neuroprotective potential of 2-aryl-N-alkylbenzimidazoles, we prepared proteins with neuroprotective functions and performed molecular docking studies to identify the neuroprotective enzymes that play a key role in metabolising 2-aryl-N-alkylbenzimidazoles. Compound 3g was listed as a model to select enzymes.

2.3.1. The Preparation of Neuroprotective Targets

The preparation began by searching for protein targets using a similarity ensemble approach (SEA) [30]. Only proteins from human and mouse samples were saved for subsequent analyses. Gene ontology (GO) annotation and cluster analysis were used as a filter for proteins with neuroprotective functions [31]. Then, 19 targets were selected for further discussion of potential targets of compound 3g. There were 14 proteins whose crystal structures were available in the Protein Data Bank (PDB) at the time of the work: APP, BMPR1B, CXCL8, CYP1B1, GLRA1, GRIK2, GRM7, HDAC6, NR4A2, PRKCG, RELA, TLR8, TOP2B, TSHR. As no crystallographic structures were available for the other five targets, CCKAR, FPR2, GABRB3, HTR5A, NPBWR1, a homology modeling methodology was used to build 3D structures of these enzymes. The appropriate template structures correspond to proteins with PDB accession codes 5ZBH, 3ODU, 4COF, 4IAR, 4N6H, respectively [32–36].

2.3.2. The Molecular Docking Simulation of 3g and Neuroprotective Targets

After the preparation of 19 neuroprotective targets, molecular docking simulations between compound 3g and neuroprotective targets were performed by use of Discovery Studio. The CHARMM force field was applied to the molecules, and CDOCKER module was used in a molecular docking
simulation [37]. The final binding results were determined by -CDOCKER ENERAGE, which means that six proteins (BMPR1B, CYP1B1, GRM7, HDAC6, HTR5A, and TOP2B) whose -CDOCKER ENERAGE is above zero are much more likely to interact with compound 3g (Figure 2a). The score of -CDOCKER ENERAGE cannot illustrate actual levels of interactions between compound 3g and the six targets. During the preliminary docking study, the major 3g-binding sites on cytochromes P450 1B1 (CYP1B1) were analyzed through comparison with the docking study of interaction between polycyclic aromatic compounds and CYP1B1. Polycyclic aromatic compounds and 2-aryl-N-alkylbenzimidazoles were found to bind to the same site (Phe231) [38]. There is no discussion about the binding mode between 2-aryl-N-alkylbenzimidazoles and CYP1B1 in the literature. As such, a molecular docking study between protein CYP1B1 and 3g was listed as a model to obtain more insight into the binding sites (Figure 2). The binding site was mainly delimited by Phe 231 through π-π interaction. The 2-naphthalenyl group also interacted with Ala330 and Ala133 residues through CH-π interaction. In addition, the naphthalene moiety in compound 3g could form a hydrogen bond with the Gly329 residue of protein CYP1B1. For the benzimidazole moiety of 3g, Val395, Ile399 and Cys470 residues were implicated in Pi bonds. The van der Waals interactions are also shown in Figure 2d. These interactions contributed to the binding affinity of 2-naphthalenyl-alkylbenzimidazole, compound 3g, and model protein CYP1B1. (Figure 2b–d) The polycyclic aromatic rings at 2-position of N-alkylbenzimidazoles seems to be important for ligands to dock into CYP1B1 through π-π interactions.

**Figure 2.** Molecular docking simulations. (a) CDOCKER ENERGY of potential targets; (b) Molecular docking of compound 3g into CYP1B1 protein; (c) Three-dimensional representation of the complex (CYP1B1 protein and 3g compound); (d) Two-dimensional interactions diagram of the complex.
3. Materials and Methods

3.1. General Methods

All reactions were carried out under a nitrogen atmosphere with oven-dried glassware. Unless otherwise stated, reagents were commercially available and used as purchased. The tetrahydrofuran (THF) was steamed through sodium to remove the aqueous part when using as a solvent, and N,N-Dimethylformamide (DMF) and xylene were dried through activated 4 Å Molecular Sieves under argon. TLC was performed with silica gel plates (GF 254) with detection under UV light at 254 nm. The NMR spectra were recorded by a Bruker 400 MHz Fourier-transform NMR spectrometer (Swiss Bruker, Chengdu, China). Chemical shifts (δ) are reported in units of parts per million (ppm) downfield from tetramethylsilane (TMS).

3.2. A Typical Procedure for the Cu-Catalyzed Arylation of Benzoimidazoles

A Schlenk 15 mL flask equipped with a magnetic bar was loaded with the benzoimidazoles with substituents at the nitrogen position (0.2 mmol) and K₂CO₃ (69.1 mg, 2.5 equiv), while CuI (10 mol %) and PPh₃ (30 mol %) were prepared in another flask. Then, the vessels were purged by a sequence of seven vacuum-argon refill-evacuation cycles. A solution of catalytic Cu/PPh₃ system was prepared through the addition of 1 mL dry DMF (or xylene) with a syringe. Preparation of the stock solution of CuI and PPh₃ was stirred for 2 h at 45 °C under argon. The catalytic solution and aryl bromide (0.3 mmol, 1.5 equiv) were added to the reaction mixture by syringe, but the solid aryl bromides were added to the reaction at the beginning. The reaction mixture was stirred for 20–44 h at 140 or 150 °C, and quenched with two drops of H₂O. The crude products were filtered over Na₂SO₄ and silica to remove salts, and rinsed with additional ethyl acetate. Finally, the solution was concentrated in vacuo. The arylated products were obtained by flash chromatography.

3.3. Preparation of Compounds 3a–3t

1-methyl-2-(naphthalen-2-yl)-1H-benzoimidazole (3a) Compound 1a (26.4 mg, 0.2 mmol) and 2-bromonaphthalene (2a, 49.6 mg, 0.24 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 24 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3a (37.3 mg), with a 72% yield rate, as a white powder; ¹H-NMR (400 MHz, CDCl₃) δ: 8.29 (1H, s), 8.00 (1H, d, J = 8.5 Hz), 7.98–7.84 (4H, m), 7.62–7.54 (2H, m), 7.46–7.41 (1H, m), 7.37–7.32 (2H, m), 3.97 (3H, s); ¹³C-NMR (400 MHz, CDCl₃) δ: 154.02, 143.32, 136.94, 133.86, 133.20, 129.58, 128.75, 128.07, 127.79, 127.44, 127.00, 126.58, 123.08, 122.75, 120.13, 109.85, 32.09. The spectroscopic data match the previously reported data [39].

3-(1-methyl-1H-benzoimidazol-2-yl)quinoline (3b) Compound 1a (16 mg, 0.1 mmol) and 3-Bromoquinoline (2b, 16 μL, 25.0 mg, 0.12 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 24 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3b (24.9 mg), with an 80% yield rate, as a white powder; ¹H-NMR (400 MHz, CDCl₃) δ: 9.34 (1H, d, J = 2.2 Hz), 8.59 (1H, d, J = 2.0 Hz), 8.20 (1H, d, J = 8.5 Hz), 7.98–7.79 (3H, m), 7.70–7.61 (1H, m), 7.45 (1H, dd, J = 6.2, 2.5 Hz), 7.42–7.33 (2H, m), 3.97 (3H, s); ¹³C-NMR (400 MHz, CDCl₃) δ: 151.12, 150.22, 148.39, 143.36, 137.08, 136.91, 131.03, 129.74, 128.56, 127.80, 127.49, 123.80, 123.10, 120.32, 110.00, 32.09; HRMS (ESI) [M + H]⁺calcd for C₁₇H₁₂N₄: 260.1188; found 260.1164.

2-(4-fluorophenyl)-1-methyl-benzoimidazole (3c) Compound 1a (16 mg, 0.12 mmol) and 4-Bromofluorobenzene (2c, 11 μL, 17.4 mg, 0.1 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 20 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3c (18.7 mg), with a 69% yield rate, as a pale yellow powder; ¹H-NMR (400 MHz, CDCl₃) δ: 7.87–7.82 (1H, m), 7.81–7.75 (2H, m), 7.42 (1H, dt, J = 4.6, 2.8 Hz), 7.39–7.32 (2H, m), 7.25 (2H, ddd, J = 11.6, 5.7, 3.6 Hz), 3.89 (3H, s); ¹³C-NMR (400 MHz, CDCl₃) δ: 165.11, 162.62, 153.01, 143.01, 136.73,
131.68, 131.60, 129.40, 123.14, 122.81, 120.04, 116.23, 116.02, 109.85, 31.88. The spectroscopic data match
the previously reported data [40].

2-(4-(tert-butyl)phenyl)-1-methyl-benzoimidazole (3d) Compound 1a (16 mg, 0.12 mmol) and 1-Bromo-4-tert-butylbenzene (2d, 17.5 µL, 21.2 mg, 0.1 mmol) were dissolved in anhydrous xylene. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3d (24.8 mg), with a 78% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl₃): δ: 7.89–7.79 (1H, m), 7.65 (4H, dd, J = 66.3, 8.4 Hz), 7.44–7.38 (m, 1H), 7.37–7.30 (2H, m), 3.90 (3H, s). 13C-NMR (400 MHz, CDCl₃): δ: 154.15, 153.21, 143.25, 136.83, 129.37, 127.51, 125.85, 122.80, 122.54, 119.98, 109.74, 35.08, 31.92, 31.46. The spectroscopic data match the previously reported data [39].

1-methyl-2-(naphthalen-1-yl)-benzoimidazole (3e) Compound 1a (16 mg, 0.12 mmol) and 1-bromonaphthalene (2e, 42.0 µL, 61.8 mg, 0.3 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3e (26.4 mg), with an 85% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl₃): δ: 8.02 (1H, d, J = 8.2 Hz), 7.98–7.86 (2H, m), 7.73 (1H, d, J = 8.3 Hz), 7.69 (1H, dd, J = 7.0, 1.1 Hz), 7.64–7.58 (1H, m), 7.57–7.51 (1H, m), 7.48 (2H, ddd, J = 9.2, 7.5, 3.0 Hz), 7.42–7.34 (2H, m), 3.62 (3H, s). 13C-NMR (400 MHz, CDCl₃): δ: 153.12, 143.39, 136.11, 133.75, 132.36, 130.51, 129.09, 128.64, 128.03, 127.41, 126.58, 125.65, 125.22, 123.02, 122.60, 120.25, 109.78, 31.26. The spectroscopic data match the previously reported data [41].

1-methyl-2-(4-trifluoromethyl)phenyl)-benzoimidazole (3f) Compound 1a (16 mg, 0.12 mmol) and 4-Bromobenzotrifluoride (2f, 39.0 µL, 67.2 mg, 0.3 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 44 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3f (23.3 mg), with a 70% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl₃): δ: 7.91 (2H, d, J = 8.2 Hz), 7.85 (1H, dd, J = 6.4, 2.5 Hz), 7.80 (2H, d, J = 8.2 Hz), 7.42 (1H, dd, J = 6.5, 2.6 Hz), 7.39–7.33 (2H, m), 3.89 (3H, s). 13C-NMR (400 MHz, CDCl₃): δ: 152.30, 143.13, 136.86, 134.01, 132.01, 130.01, 125.91, 125.88, 125.59, 123.05, 120.35, 109.99, 31.97. The spectroscopic data match the previously reported data [39].

2-(4-methoxyphenyl)-1-methyl-benzoimidazole (3g) Compound 1a (16 mg, 0.12 mmol) and 2-Bromobenzotrifluoride (2g, 71.7 mg, 0.3 mmol) were dissolved in anhydrous xylene. The solution was stirred at 140 °C for 40 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3g (25.0 mg), with a 72% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl₃): δ: 8.17 (1H, s), 7.91–7.80 (4H, m), 7.44–7.37 (1H, m), 7.35–7.28 (2H, m), 7.25–7.16 (2H, m), 3.93 (6H, d, J = 16.2 Hz). 13C-NMR (400 MHz, CDCl₃): δ: 158.92, 154.21, 143.27, 136.90, 135.23, 130.24, 129.32, 128.64, 127.42, 127.09, 125.46, 122.89, 122.62, 119.95, 119.89, 109.77, 105.93, 55.59, 32.04; HRMS (ESI) [M + H]+ calc'd for C₁₉H₁₇N₂O: 289.1341; found 289.1344.

1-methyl-2-(thiophen-2-yl)-benzoimidazole (3h) Compound 1a (16 mg, 0.12 mmol) and 2-Bromothiophene (2h, 29.0 µL, 49.0 mg, 0.3 mmol) were dissolved in anhydrous xylene. The solution was stirred at 140 °C for 40 h. The residue was purified by column chromatography (hexane/ EtOAc = 5:1) to afford 3h (18.2 mg), with a 70% yield rate, as a green and yellow powder; 1H-NMR (400 MHz, CDCl₃): δ: 7.80 (1H, dd, J = 6.2, 2.7 Hz), 7.53 (2H, dd, J = 17.8, 4.1 Hz), 7.36–7.27 (3H, m), 7.18 (1H, dd, J = 4.8, 3.9 Hz), 3.95 (3H, s). 13C-NMR (400 MHz, CDCl₃): δ: 148.03, 143.00, 136.72, 132.68, 128.68, 128.10, 127.97, 127.09, 122.79, 119.86, 109.55, 31.78. The spectroscopic data match the previously reported data [39].

1-methyl-2-(pyridin-2-yl)-benzoimidazole (3i) Compound 1a (16 mg, 0.12 mmol) and 2-Bromopyridine (2i, 33.0 µL, 69.1 mg, 0.3 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/ EtOAc = 5:1) to afford 3i (13.6 mg), with a 54% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl₃): δ: 8.69 (1H, d, J = 4.3 Hz), 8.38 (1H, d, J = 8.0 Hz), 7.88–7.80 (2H, m), 7.43 (1H, d, J = 7.3 Hz), 7.38–7.28 (3H, m), 4.27 (3H,
2-(4-fluorophenyl)-1-ethyl-benzoimidazole (3j) Compound 1b (35.4 mg, 0.2 mmol) and 4-Bromofluorobenzene (2j, 33.0 µL, 52.2 mg, 0.3 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3j (20.1 mg), with a 42% yield rate, as a yellow powder; 1H-NMR (400 MHz, CDCl3) δ: 7.85 (1H, dd, J = 5.1, 3.9 Hz), 7.78–7.71 (2H, m), 7.49–7.42 (1H, m), 7.39–7.30 (2H, m), 7.26 (2H, dd, J = 15.8, 7.2 Hz), 4.30 (2H, q, J = 7.2 Hz), 1.48 (3H, t, J = 15.5, 8.2 Hz). 13C-NMR (400 MHz, CDCl3) δ: 165.09, 162.60, 152.67, 143.30, 135.54, 131.48, 131.39, 129.48, 126.96, 126.93, 123.04, 122.69, 120.20, 116.25, 116.03, 110.13, 92.63, 39.81, 29.90; HRMS (ESI) [M + H]+ calcd for C15H14FN2: 241.1141; found 241.1119.

1-ethyl-2-(thiophen-2-yl)-benzoimidazole (3n) Compound 1b (35.4 mg, 0.2 mmol) and 2-Bromonaphthalene (2a, 62.3 mg, 0.3 mmol) were dissolved in anhydrous xylene. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3n (21.6 mg), with a 40% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl3) δ: 8.24 (1H, s), 8.00 (1H, d, J = 8.5 Hz), 7.94 (2H, t, J = 8.7 Hz), 7.90–7.81 (2H, m), 7.62–7.54 (2H, m), 7.50–7.43 (1H, m), 7.38–7.30 (2H, m), 4.37 (2H, q, J = 7.2 Hz), 1.51 (3H, t, J = 7.2 Hz). 13C-NMR (400 MHz, CDCl3) δ: 153.69, 143.57, 135.73, 133.87, 133.23, 129.37, 128.75, 128.72, 128.15, 128.06, 127.40, 126.98, 126.41, 122.64, 120.26, 110.15, 39.96, 15.54; HRMS (ESI) [M + H]+ calcd for C19H17N2: 273.1392; found 273.1344.

1-ethyl-2-(naphthalen-2-yl)-1H-benzoimidazole (3o) Compound 1c (44.4 mg, 0.2 mmol) and 2-Bromonaphthalene (2a, 62.3 mg, 0.3 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/EtOAc = 5:1) to afford 3o (32.2 mg), with a 50% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl3) δ: 8.16
1-benzyl-2-(4-(trifluoromethyl)phenyl)-benzoimidazole (3p) Compound 1c (44.4 mg, 0.2 mmol) and 4-Bromofluorobenzene (1f, 30.0 µL, 67.2 mg, 0.3 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/ETOAc = 5:1) to afford 3p (30.3 mg), with a 43% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl3) δ: 7.93 (1H, d, J = 8.0 Hz), 7.85 (2H, d, J = 8.1 Hz), 7.73 (2H, d, J = 8.2 Hz), 7.41–7.29 (6H, m), 7.13 (2H, dd, J = 9.3, 7.8 Hz), 5.49 (2H, s). 13C-NMR (400 MHz, CDCl3) δ: 123.90, 143.27, 136.42, 136.22, 133.79, 129.80, 129.47, 129.33, 128.78, 128.77, 128.06, 128.00, 127.57, 127.45, 126.91, 126.39, 126.26, 123.36, 122.98, 120.25, 110.70, 48.79. The spectroscopic data match the previously reported data [43].

1-benzyl-2-(4-fluorophenyl)-benzoimidazole (3q) Compound 1c (44.4 mg, 0.2 mmol) and 50% yield rate, as a white powder; H-NMR (400 MHz, CDCl3) δ: 7.90–7.79 (2H, m), 7.38–7.32 (4H, m), 7.31–7.25 (2H, m), 7.16 (4H, ddd, J = 15.5, 9.2, 4.7 Hz), 5.46 (2H, s); 13C-NMR (400 MHz, CDCl3) δ: 156.18, 162.69, 153.37, 143.27, 136.45, 136.29, 131.50, 131.41, 129.33, 128.08, 126.08, 123.38, 122.99, 120.19, 116.24, 116.03, 110.66, 48.54. The spectroscopic data match the previously reported data [43].

1-benzyl-2-(pyridin-2-yl)-benzoimidazole (3r) Compound 1c (44.4 mg, 0.2 mmol) and 2-Bromopyridine (2i, 33.0 µL, 150.50, 149.94, 147.40, 143.00, 137.92, 137.41, 136.81, 129.96, 128.76, 127.53, 127.00, 124.90, 124.04, 123.76, 122.99, 120.34, 110.96, 49.11. The spectroscopic data match the previously reported data [44].

3-(1-benzyl-1H-benzo[d]imidazol-2-yl)quinoline (3s) Compound 1c (44.4 mg, 0.2 mmol) and 3-Bromoquinoline (3t, 44.4 mg, 0.2 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/ETOAc = 5:1) to afford 3s (32.5 mg) with a 54% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl3) δ: 7.89 (1H, d, J = 8.0 Hz), 7.74–7.66 (2H, m), 7.38–7.32 (4H, m), 7.31–7.25 (2H, m), 7.16 (4H, ddd, J = 15.5, 9.2, 4.7 Hz), 5.46 (2H, s); 13C-NMR (400 MHz, CDCl3) δ: 156.18, 162.69, 153.37, 143.27, 136.45, 136.29, 131.50, 131.41, 129.33, 128.08, 126.08, 123.38, 122.99, 120.19, 116.24, 116.03, 110.66, 48.54. The spectroscopic data match the previously reported data [44].

3-(1-benzylbenzoimidazol-2-yl)isoquinoline (3t) Compound 1c (44.4 mg, 0.2 mmol) and 2-Bromoquinoline (2t, 32.0 µL, 50.2 mg, 0.24 mmol) were dissolved in anhydrous DMF. The solution was stirred at 150 °C for 40 h. The residue was purified by column chromatography (hexane/ETOAc = 5:1) to afford 3t (34.0 mg), with a 53% yield rate, as a white powder; 1H-NMR (400 MHz, CDCl3) δ: 9.26 (1H, d, J = 2.2 Hz), 8.46 (1H, d, J = 2.0 Hz), 8.16 (1H, dd, J = 8.4, 0.7 Hz), 7.94 (1H, d, J = 8.0 Hz), 7.80 (2H, ddd, J = 9.2, 5.4, 2.0 Hz), 7.60 (1H, ddd, J = 8.1, 6.9, 1.1 Hz), 7.40–7.31 (6H, m), 7.14 (2H, d, J = 6.9 Hz), 5.54 (2H, s); 13C-NMR (400 MHz, CDCl3) δ: 151.48, 150.09, 148.46, 143.51, 136.96, 136.58, 136.28, 131.05, 129.66, 129.50, 128.60, 128.32, 127.72, 127.36, 126.08, 123.90, 123.62, 123.33, 120.46, 110.73, 48.76; HRMS (ESI) [M + H]+ calcd for C23H18F4N3: 336.1501; found 336.1500.
3.4. Determination of Protective Rate by MTT Assay

Cells were cultured in 96-well plates with a density of $5 \times 10^3$ cells for one day, and then pre-treated at 37 °C for 4 h with compounds 3b, 3g, 3h, 3i, 3j, 3k, 3o, 3q, 3s, and 3t (10 and 50 µM, $n = 3$). The cells were treated with 1nm MPP$^+$ in the continued presence of low-neurotoxicity compounds for 24 h, and then assessed for cell viability. Cell viability was determined by a mitochondrial enzyme dependent reaction of MTT assay. In brief, 10 µL of MTT 5 mg/mL was added directly to each culture media with SH-SY5Y cells. Following a two-hour incubation at 37 °C, the supernatants were removed, and then 150 µL solubilizing agent was added to dissolve the purple formazan crystals formed by metabolism of the yellow MTT tetrazolium salt. The plate was then incubated for 1 h or overnight, and the absorbance was measured using a microplate reader (Wellscan MK3, Labsystems Dragon) at a wavelength of 570 nm. Cell death following MPP$^+$ treatment was shown by the inhibition rate of untreated cells. Microsoft® Excel 2010 was used to analyze data. The neuroprotective effects by the compounds were expressed as the protective rate, and it was calculated according to the following formulas:

Inhibition rate = \frac{(mean OD_{control} − mean OD_{drug reated})}{(mean OD_{control} − mean OD_{vacuity})} \times 100%

Cell viability = 1 − Inhibition rate

Protective rate = \frac{(Cell viability_{drug reated} − Cell viability_{MPP^+})}{Cell viability_{MPP^+}} \times 100%

And Cell viability_{MPP^+} = 46.35%

3.5. A Procedure for the Preparation of Neuroprotective Targets

Relying on the structural similarity, possible targets related to 3g were predicted by the similarity ensemble approach (SEA) (http://sea.bkslab.org/) [30]. After the screening of targets, only proteins from human and mouse were continuously analyzed through gene ontology (GO) annotation and cluster analysis. According to the results from GO annotation and cluster analysis, the following six results related to neuroprotective function (inhibitory postsynaptic potential, cellular response to hydrogen peroxide, chemical synaptic transmission, inflammatory response, adult locomotory behavior, and axonogenesis) were obtained, and 19 targets were selected [31]. Among them, the 3D crystal structures of 14 targets were retrieved from the Protein Data Bank (PDB); their names and PDB codes were APP (PDB ID: 3KTM), BMPR1B (PDB ID: 3MDY), CXCL8 (PDB ID: 5PM0), GLRA1 (PDB ID: 3JAF), GRIK2 (PDB ID: 5CMM), GRM7 (PDB ID: 3MP4), HDAC6 (PDB ID: 6CE6), NR4A2 (PDB ID: 1OVL), PRKCG (PDB ID: 2UZP), RELA (PDB ID: 3GUT), TLR8 (PDB ID: 3W3J), TOP2B (PDB ID: 4G0W), TSHR (PDB ID: 3G04). The 3D structures of another 5 proteins, CCKAR, FPR2, GABRB3, HTR5A, NPBWR1, that were absent from this study were constructed by homology modeling using SWISS-MODEL. This protein structure homology-modeling server is one of the most accurate computational methods (https://swissmodel.expasy.org/workspace/) [33–37]. The template structures for CCKAR, FPR2, GABRB3, HTR5A, and NPBWR1 were downloaded from PDB for the homology methodology; their template codes are 5ZBH, 3ODU, 4COF, 4IAR, 4N6H, respectively (The results of SEA, GO annotation, cluster analysis, homology modeling and the summarizing scheme of in silico studies are shown in the Supplementary Materials).

3.6. A Procedure for Molecular Docking Simulation

The Accelrys Discovery Studio docking program (version 3.5, Accelrys Inc., Bedminster Township, NJ, USA) was adopted here [32]. For the module, CDOCKER is a molecular dynamics (MD) simulated-annealing-based algorithm, and ligand conformations are generated using a high-temperature molecular dynamics methodology and energy minimized into the protein active site using simulated annealing molecular dynamics [45]. Although the docking accuracy decreases when ligands with eight or more rotatable bonds, low-flexible 2-aryl-N-alkylbenzimidazoles could have appropriate docking accuracy rates [37,46]. In this program, the preparations of protein structures included adding...
hydrogen atoms, removing water molecules, and assigning a CHARMM forcefield. After clicking the CDOCKER module, the input receptors were set up as the corresponding targets, and the input ligand was set up as compound 3g. Top hits were selected as 10, and the other parameters were set as default.

4. Conclusions

We demonstrated a copper-catalyzed direct arylation of N-alkylbenzimidazoles with diverse aryl bromides to furnish the corresponding 2-aryl-N-alkylbenzimidazoles. All these compounds were evaluated for their in vitro neurotoxicities, but low-neurotoxic compounds (3b, 3g, 3h, 3i, 3j, 3k, 3o, 3q, 3s and 3t) were found to exert a low neuroprotective effect. This preliminary study provided basic research about 2-aryl-N-alkylbenzimidazoles based on neurodegenerative diseases. Six targets, BMPR1B, CYP1B1, GRM7, HDAC6, HTR5A, and TOP2B, could be potential goals for the study of 2-aryl-N-alkylbenzimidazoles as neuroprotective agents. The dicyclic aromatic rings at the 2-position of N-alkylbenzimidazoles could be important structure moieties for the interaction with CYP1B1, according to our atomic-level study of the interaction between 3g and CYP1B1. This work generated a series of compounds for the 2-arylbenzimidazoles library using a direct protocol, and provided a basic evaluation of 2-aryl-N-alkylbenzimidazoles as neuroprotective agents. Six potential targets and suggestions about the structure of 2-aryl-N-alkyl benzimidazoles as a ligand for CYP1B1 were provided in terms of atomic level.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4344/8/10/433/s1.

Author Contributions: Conceptualization, F.G. and X.-X.L.; methodology, Y.-X.Y.; software, Y.-X.Y.; validation, N.-N.J.; Y.-N.C and X.-X.C.; formal analysis, Y.-X.Y.; investigation, N.-N.J.; resources, Y.-N.C.; data curation, Y.-X.Y.; writing—original draft preparation, Y.-X.Y.; writing—review and editing, F.G.; visualization, Y.-X.Y.; supervision, F.G.; project administration, F.G.; funding acquisition, F.G.

Funding: This research was funded from NSFC (grant number 31570341) and Key Technology Program from Sichuan Province, China (grant number 2015SZ0105).

Acknowledgments: I acknowledge the support of Key Technology Program from Sichuan Province and help of Guangchuan Wang and Shuangmei Zhao.

Conflicts of Interest: The authors declare no conflict of interest.

References
5. Pinna, A. Adenosine A2A Receptor Antagonists in Parkinson’s Disease: Progress in Clinical Trials from the Newly Approved Istradefylline to Drugs in Early Development and Those Already Discontinued. CNS Drugs 2014, 28, 455–474. [CrossRef] [PubMed]

26. Zhang, W.; Tian, Y.; Zhao, N.; Wang, Y.; Li, J.; Wang, Z. Nano CuO-catalyzed C–H functionalization of...

23. Do, H.Q.; Daugulis, O. Copper-catalyzed arylation of heterocycle C-H bonds.


36. Peitsch, M.C. Protein Modeling by E-mail. Biotechnology 1995, 13, 658–660. [CrossRef]


