Synthesis and SAR study of diphenylbutylpiperidines as cell autophagy inducers

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Abstract

A novel series of diphenylbutylpiperidines as autophagy inducers was described and extensive SAR studies resulted in derivatives (15d–e, 15i–j) with 10-fold greater activity than the lead compounds 1 and 2. Meanwhile, a new synthetic route to diphenylbutyl bromide (6) from bromobenzene and y-butyrolactone was also reported here.

Keywords:
Autophagy
Diphenylbutylpiperidines
Synthesis
SAR study
Inducers

Autophagy, a cellular pathway involved in protein and organelle turnover, has been shown to play an important role in human physiology and various diseases such as cancer, cardiomyopathy and neurodegenerative disorders (Alzheimer’s, Parkinson’s, and Huntington’s diseases, amyotrophic lateral sclerosis and prion diseases) [1].

Due to its importance, the regulation of autophagy under starvation has been extensively studied. For example, as the target of rapamycin in mammalian cells, mTOR kinase mediates a major inhibitory signal that shuts off autophagy under nutrient-rich conditions. Therefore, inhibition of mTOR can lead to the activation of autophagy in response to starvation. Moreover, autophagy plays an important role in cells under normal nutritional conditions by mediating protein turnovers and loss of autophagy function in the nervous systems [2]. However, the regulation of autophagy under normal nutritional conditions still remains unknown in large and has been a great challenge to scientists ever since.

In our ongoing projects, we are interested in the design and synthesis of biologically active small molecules that can induce autophagy. In our initial studies, a high-throughput image-based screen was carried out and seven FDA-approved drugs were found to induce autophagy without causing cell death [3]. It is interesting that five of these compounds have been known to inhibit the intracellular Ca2+ flux and discovered that calpains play an important role in controlling the levels of autophagy in normal living cells by regulating the levels of ATG5 [4].

Recently, we also confirmed the activity of fluspirilene in inhibiting Ca2+ flux and discovered that calpains play an important role in controlling the levels of autophagy in normal living cells by regulating the levels of ATG5 [5].

It is worthy to point out that three of these identified autophagy inducers (Fig. 1), fluspirilene, Pimozide, and Trifluoperazine, are all derivatives of diphenylbutylpiperidines (DPBPs). This class of compounds, which were originally developed as antagonists of the D2 receptor, are now used clinically to treat various forms of psychosis [6]. Furthermore, recent evidence suggests that DPBPs are also potent antagonists of calcium channels [7]. In addition, Penfluridol, one of the common DPBPs, which is not present in the library screened, was discovered as a good autophagy inducer (EC50 = 3.2 μM) as well.

Encouraged by these results, we began to switch our attention onto the structural modification of diphenylbutylpiperidines. Herein, we would like to report our recent SAR study of a new family of DPBPs as cell autophagy inducers.

All of the DPBPs analogs described in this Letter were prepared by nucleophilic substitution of diphenylbutyl halide with piperidines according to literature [8]. As a key intermediate of DPBPs, diphenylbutyl bromide was synthesized via various routes [8,9]. However, among these methods, relatively expensive as well as the limited variety starting material forced us to find a more effective access. As shown in Scheme 1, treatment of Grignard reagents onto the structural modification of diphenylbutylpiperidines. Herein, we would like to report our recent SAR study of a new family of DPBPs as cell autophagy inducers.

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Various structures of DPBPs could be obtained by simple transformations.

Unlike the synthetic strategy above, DPBPs compounds with tricyclic system were prepared as outlined in Scheme 2. Grignard reaction of bromocyclopropane with tricyclic ketone afforded compound 7, which then rearranged to compound 8 by magnesium bromide. Subsequent hydrogenation of 8 gave the target molecule 9.

Considering that there is a tertiary hydroxyl group in the 4-position of piperidine ring, which makes the structure of this type of compounds unique compared to others, our efforts were then exerted on the synthesis of its derivatives. As shown in Scheme 3 and 4, Grignard reaction of bromobenzene derivatives with N-Boc piperidone gave corresponding compound 10. However, removal of Boc group of 10 with trifluoroacetic acid only led to the dehydrated products. After several trials, piperidine 11 could be synthesized from 10 in the mixture of 3 M aqueous HCl and ethyl acetate at room temperature. Subsequent protection of hydroxyl group of resulting molecules 17a–f, j by acetyl group gave compounds 18a–g.8

With these compounds in hand, we began to estimate them as follows: H4-LC3 cells were cultured in the presence of indicated compounds for 4 h, fixed with 4% paraformaldehyde (Sigma) and stained with 3 μg/ml DAPI (Sigma). Images data were collected.
with an ArrayScan HCS 4.0 Reader with a 20× objective (Cellomics) for DAPI-labeled nuclei and GFP-tagged intracellular proteins. The Spot Detector BioApplication was used to acquire and analyze the images after optimization. Images of 1000 cells for each compound treatment were analyzed to obtain the average cell number per field, fluorescence spot number, area, and intensity per cell. The EC$_{50}$ was analyzed using GraphPad Prism 4. DMSO and rapamycin were used as negative or positive control, respectively. The percentages of changes of LC3-GFP were calculated by dividing with that of DMSO-treated samples. Each treatment was done in triplicate to obtain the mean ± SD. The images were also analyzed by using a conventional fluorescence microscope for visual inspection. The experiments were repeated three times with consistent results.

As shown in Table 1, the impact of the modification of the left-hand sides on the cell autophagy inducing activity is well studied.

<table>
<thead>
<tr>
<th>R</th>
<th>Compound/EC$_{50}$ (µM)</th>
<th>Compound/EC$_{50}$ (µM)</th>
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<tbody>
<tr>
<td></td>
<td>12a (1)/2.4</td>
<td>13a (2)/3.2</td>
</tr>
<tr>
<td></td>
<td>12b/14.8</td>
<td>13b/3.2</td>
</tr>
<tr>
<td></td>
<td>12c/24.9</td>
<td>13c/4.3</td>
</tr>
<tr>
<td></td>
<td>12d/6.2</td>
<td>13d/&gt;50</td>
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<tr>
<td></td>
<td>12e/&gt;50</td>
<td>13e/&gt;50</td>
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<td>12f/&gt;50</td>
<td>13f/&gt;50</td>
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<tr>
<td></td>
<td>12g/&gt;50</td>
<td>13g/&gt;50</td>
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<tr>
<td></td>
<td>12h/&gt;50</td>
<td>13h/&gt;50</td>
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Firstly, changing the linker of the diphenyl ring and alkyl chain (12b, 12c, and 12d) leads to the decrease of bioactivity, indicating that appropriate space is needed. Interestingly, compound 13b (CN vs H) showed the same strength of bioactivity as compound 13a. Secondly, compounds 12e and 13d were inactive, which can be rationalized by the fact that the amide linkage could be easily hydrolyzed in vivo. Thirdly, compounds 12f-i with one phenyl ring removed showed no activity, indicating the importance of the diphenyl part. Lastly, we also changed the lengths of the spacer linkers. Compounds 12j and 13e, with one more methylene group compared with compounds 12a and 13a, demonstrated twice weaker bioactivity of 12j but a little more activity of 13e.

In contrast, one less methylene group gave no change of activity (12k), while compound 12f showed 1.5-fold decreased activity. These results indicated that there may be some tolerance when it comes to the space length (2–4 carbon chain).

In addition, the impact of substituents on the diphenyl ring was examined as a part of the SAR studies. As shown in Table 2, compounds 14a and 15a with no substituent on the diphenyl ring

| Table 2 |
| EC50 induction for diphenyl ring substitution |

<table>
<thead>
<tr>
<th>R</th>
<th>Compound/EC50 (µM)</th>
<th>Compound/EC50 (µM)</th>
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<tbody>
<tr>
<td>H</td>
<td>14a/3.46</td>
<td>15a/5.1</td>
</tr>
<tr>
<td>4-F</td>
<td>14b/1.1</td>
<td>15b/2.4</td>
</tr>
<tr>
<td>4-Cl</td>
<td>14c/4.2</td>
<td>15c/2.4</td>
</tr>
<tr>
<td>4-CF3</td>
<td>14d/3.7</td>
<td>15d/0.28</td>
</tr>
<tr>
<td>4-CH3</td>
<td>14e/3.1</td>
<td>15e/0.29</td>
</tr>
<tr>
<td>4-OCH3</td>
<td>14f/4.6</td>
<td>15f/1.2</td>
</tr>
<tr>
<td>4-OCF3</td>
<td>–</td>
<td>15g/0.97</td>
</tr>
<tr>
<td>3-F</td>
<td>–</td>
<td>15h/2.2</td>
</tr>
<tr>
<td>3-CF3</td>
<td>–</td>
<td>15i/0.63</td>
</tr>
<tr>
<td>3-CH3</td>
<td>–</td>
<td>15j/0.29</td>
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</tbody>
</table>

| Table 3 |
| EC50 induction for different tricyclic moieties |

<table>
<thead>
<tr>
<th>R</th>
<th>Compound/EC50 (µM)</th>
<th>Compound/EC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>14g/7.9</td>
<td>15k/2.6</td>
</tr>
<tr>
<td>4-F</td>
<td>14h/&gt;50</td>
<td>15l/14.4</td>
</tr>
<tr>
<td>4-Cl</td>
<td>14i/5.1</td>
<td>15m/2.6</td>
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<p>| Table 1 (continued) |</p>
<table>
<thead>
<tr>
<th>R</th>
<th>Compound/EC50 (µM)</th>
<th>Compound/EC50 (µM)</th>
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</thead>
<tbody>
<tr>
<td>F</td>
<td>12i/&gt;50</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>12j/4.7</td>
<td>13e/2.7</td>
</tr>
<tr>
<td>F</td>
<td>12k/2.1</td>
<td>13f/4.5</td>
</tr>
</tbody>
</table>

| 237 |
demonstrated the lowest activity in this series. On the other hand, compounds 14c–f with para-substituted diphenyl ring still showed no remarkable improvement in activity compared with compound 14b. By contrast, compounds 15c–j with various para or meta-substituents exhibited promising results. Among them, compound 15d (R = 4-CF₃), 15e (R = 4-CH₃), and 15j (R = 3-CH₃) were ten times more potent when compared with compound 15b, and compound 15i (R = 3-CF₃) showed fivefold improved bioactivity. On the whole, the electronic effect of substituents on the diphenyl has no significant effect on the bioactivity (15d vs 15e, 15i vs 15j). These results suggest that appropriate steric hindrance on the diphenyl ring will exert a positive effect on the bioactivity.

As showed in Table 3, incorporation of the two phenyl rings in tricyclic moieties which brought more rigidity, afforded different results. Compounds 14g and 14i showed lower activity compared with compound 14b. However, for compounds 15k and 15m, the bioactivity enhanced a little bit. Probably due to the poor solubility when a sulfur atom was introduced, compound 14h lost bioactivity completely while compound 15l showed less activity.

After the modification of the left-hand sides of DPBPs, we decided to examine the effects of the piperidine ring on the bioactivity. As shown in Table 4, compounds 16a–c with a piperazine ring as well as compound 16d with a morpholine ring lacked activity completely. Compound 16e with an unsaturated piperidine and 16f with no hydroxyl group also showed no inducing activity. As previously reported in the literature, changing of hydroxyl group, either protected by acetyl group (16h) or substituted with acetamide group (16i), showed good results. However, compounds 16j with cyano-group instead of the hydroxyl group showed tiny autophagy inducing activity. These results above indicated that the six-membered ring in the right side need appropriate conformation and the hydroxyl group or related groups may interact with the target protein through hydrogen bonding.

Encouraged by the results of Table 4, we changed substituents on the piperidine phenyl ring and groups at R₁ to examine their effects on the bioactivity. As shown in Table 5, when R₁ = OH, compounds 17b, 17e–f with para-substituted on the phenyl ring showed better results than compounds 17g–i with meta-substituents, while

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>EC₅₀ (µM)</th>
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<tbody>
<tr>
<td>16a</td>
<td></td>
<td>&gt;50</td>
</tr>
<tr>
<td>16b</td>
<td></td>
<td>&gt;50</td>
</tr>
<tr>
<td>16c</td>
<td></td>
<td>&gt;50</td>
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<td>16d</td>
<td></td>
<td>&gt;50</td>
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<td>16e</td>
<td></td>
<td>&gt;50</td>
</tr>
<tr>
<td>16f</td>
<td></td>
<td>&gt;50</td>
</tr>
<tr>
<td>16g</td>
<td></td>
<td>6.4</td>
</tr>
<tr>
<td>16h</td>
<td></td>
<td>4.4</td>
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<tr>
<td>16i</td>
<td></td>
<td>2.2</td>
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<tr>
<td>16j</td>
<td></td>
<td>10.5</td>
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</table>
compound 17a with no substituent on the phenyl ring showed decreased bioactivity.

Since it has been found that the presence of a hydroxyl group at 4-position of the piperidine ring may lead to potential metabolic toxicity, introduction of an ester group and amide group was then achieved to eliminate the negative effect (Table 5). Unfortunately, 18a–f showed no improved activity when compared with 17a–f. On the other hand encouraging results were obtained when an amide group was introduced (19a–f). However, both compounds 18g and 19g displayed decreased inducing activity compared with compound 17j, indicating appropriate space on the piperidine ring is responsible to the activity of compound under investigation.

In summary, we have identified a novel class of diphenylbutyl amides with no substituent on the phenyl ring is responsible to the activity of compound under investigation.

**Acknowledgments**

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**Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.029.

**References and notes**

11. For the synthesis of the left side of compound 12b, see Ref.: Kulp, S. S.; Fish, V. B.; Easton, N. R. J. Med. Chem. 1963, 6, 516.
12. The structure of the left side of compound 12d is the same as compound 5 (R = 4-F).
13. The carboxylic acid group in the left of compound 12e can be prepared from compound 4 (R = 4-F) by hydrogenation, Jones oxidation.
15. The left side of compound 12b can be synthesized from the same route as shown in Scheme 1 with the starting material s-valeronolactone instead of γ-butyrolactone.
17. In this Letter we mainly used the left side with three carbon chain due to its easy preparation.
18. The right side of compound 16b can be prepared from compound 10 (R = CH3) by removing the Boc group with trifluoroacetic acid, and hydrogenation of compound 16c can give compound 16d.
19. The right side of compound 16e is commercially available.