Bioorganic & Medicinal Chemistry Letters 21 (2011) 234–239

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Synthesis and SAR study of diphenylbutylpiperidines as cell autophagy inducers

Gang Chen [a], Hongguang Xia [a], Yu Cai [a], Dawei Ma [a,*], Junying Yuan [a,b,*], Chengye Yuan [a,*]

[a] State Key Laboratory of Bio-organic and Natural Product Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China
[b] Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA

ARTICLE INFO

*Article history:*
Received 23 July 2010
Revised 10 October 2010
Accepted 4 November 2010
Available online 11 November 2010

*Keywords:*
Autophagy
Diphenylbutylpiperidines
Synthesis
SAR study
Inducers

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 γ-butyrolactone was also reported here.



Autophagy, a cellular pathway involved in protein and organelle turnover, has been proved to play an important role in human physiology and several 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 $Ca^{2+}$ for decades. It should be noted that previous studies have shown the possible involvement of intracellular $Ca^{2+}$ in regulating autophagy. For example, L-type $Ca^{2+}$ channel antagonists, the $K^+$ATP channel opener minoxidil, and the Gi signaling activator clonidine could induce autophagy in mTOR-independent manner.[4] Recently, we also confirmed the activity of fluspirilene in inhibiting $Ca^{2+}$ 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 $D_2$ 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 ($EC_{50}$ = 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 of bromobenzene derivatives with γ-butyrolactone gave dihydroxyl compound **3**, which was then converted to dehydrated compound **4** in acidic conditions. Compound **6** was synthesized from **4** through bromination followed by hydrogenation. In this way,

* Corresponding authors. Fax: +86 21 5492 5379.
*E-mail address:* yuancy@mail.sioc.ac.cn (C. Yuan).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.11.029

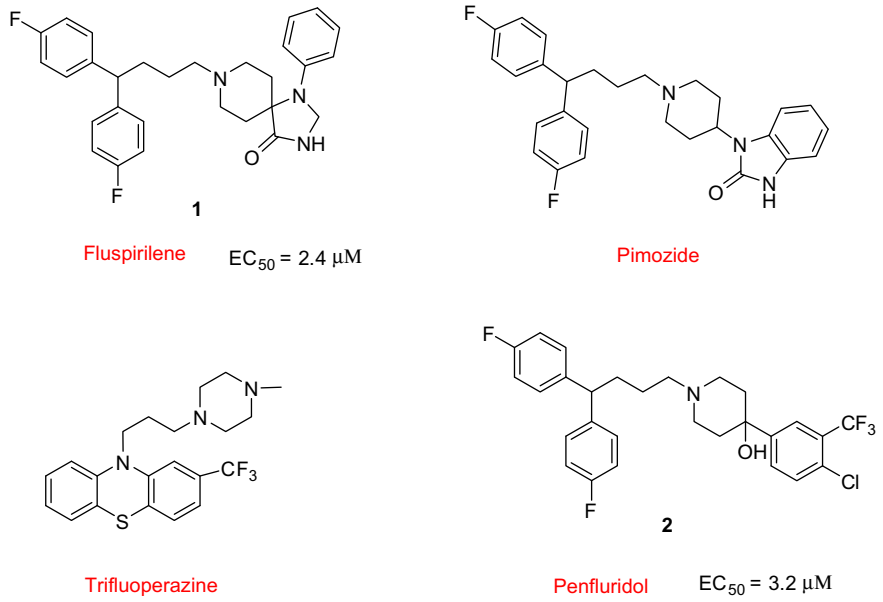
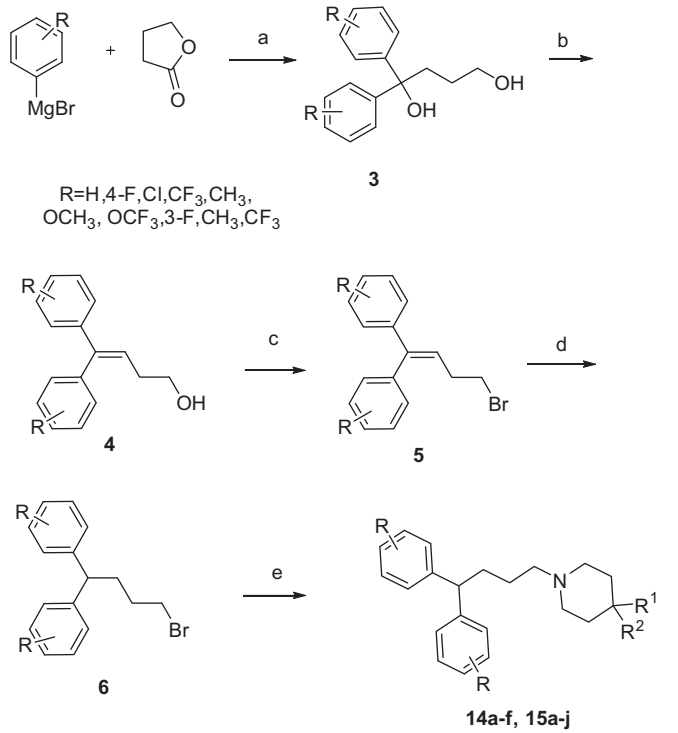
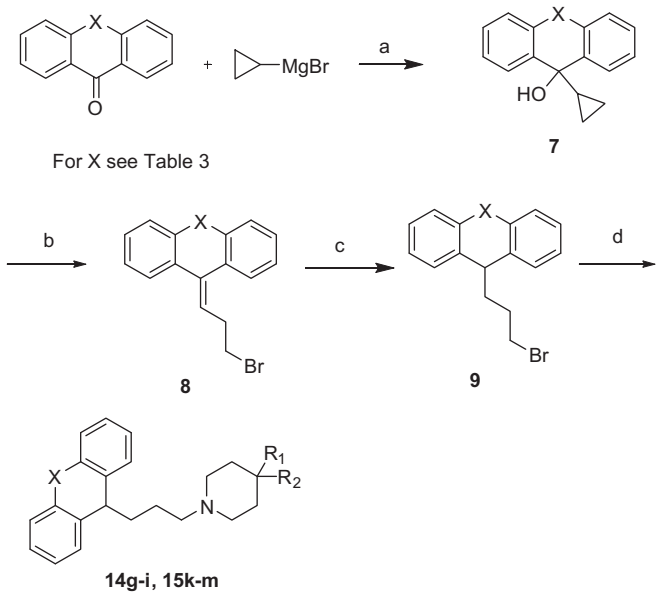



**Figure 1.** DPBPs autophagy regulators.




**Scheme 1.** Reagents and conditions: (a) THF, reflux; (b) EtOH, cond HCl, reflux; (c) $CBr_4$, $Ph_3P$, $CH_2Cl_2$, 0 °C to rt; (d) Pd–C, $H_2$, EtOH, rt; (e) piperidines, $Na_2CO_3$, Kl, $CH_3CN$, reflux.



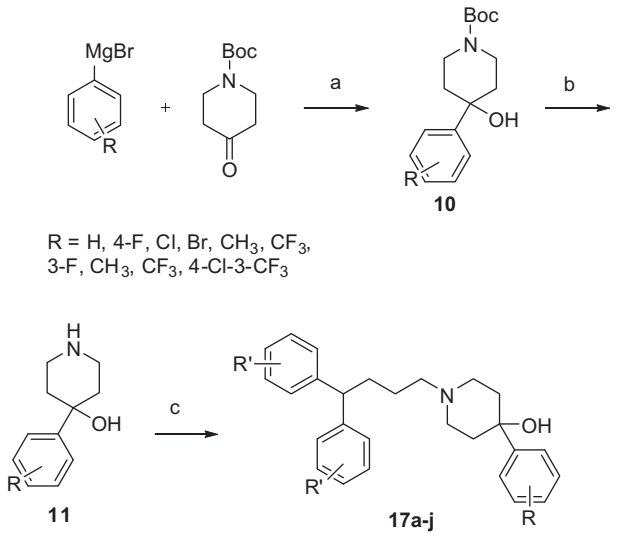

**Scheme 2.** Reagents and conditions: (a) EtOH, reflux; (b) $MgBr_2$, $Et_2O$, reflux; (c) Pd–C, $H_2$, EtOH, rt; (d) piperidines, $Na_2CO_3$, Kl, $CH_3CN$, reflux.

various structures of DPBPs could be obtained by simple transformations.

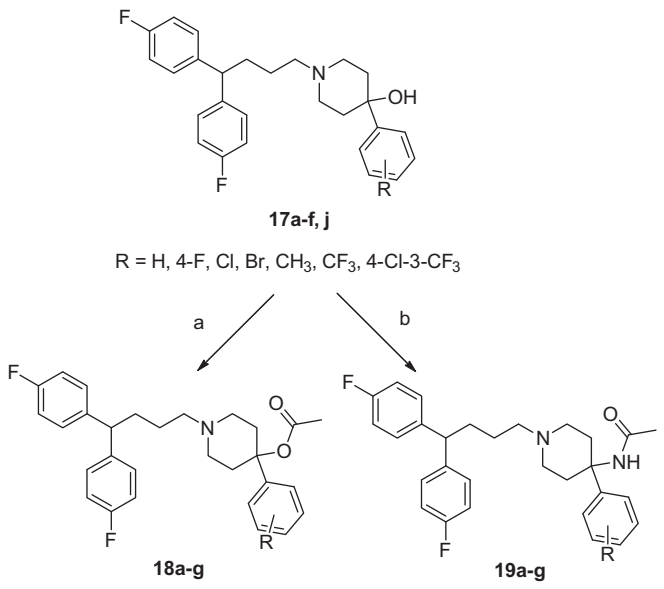
Unlike the synthetic strategy above, DPBPs compounds with tricyclic system were prepared as outlined in Scheme 2. Grignard reaction of bromocyclopropane with tricyclic ketone afforded compound **7**, which then rearranged to compound **8** by magnesium bromide. Subsequent hydrogenation of **8** gave 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**[10] and substitution with acetamide group by Ritter reaction provided corresponding compounds **19a–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




**Scheme 3.** Reagents and conditions: (a) THF, reflux; (b) EtOAc, 3 M HCl rt; (c) diphenylbutyl bromide, $Na_2CO_3$, KI, $CH_3CN$, reflux.

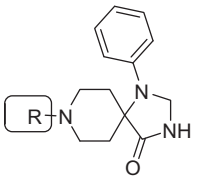


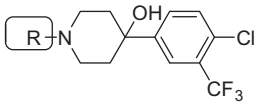

**Scheme 4.** Reagents and conditions: (a) acetyl chloride, $CHCl_3$, 0 °C to rt; (b) concd $H_2SO_4$, $CH_3CN$, rt.

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 1**
$EC_{50}$ induction for left-hand side modification



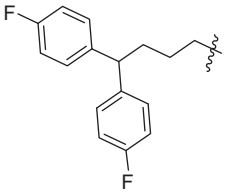
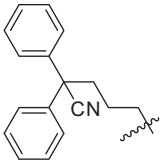
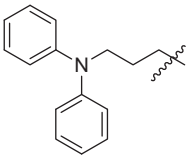
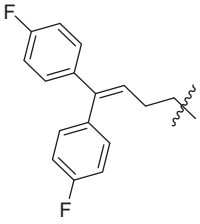
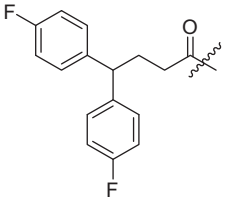
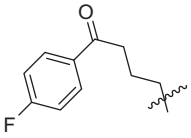
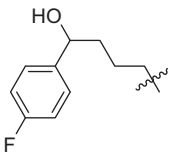
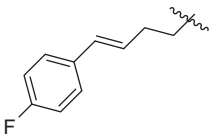
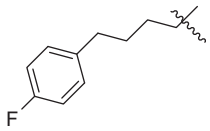
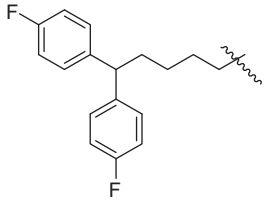
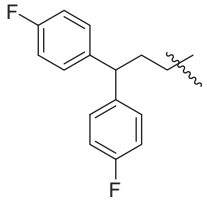
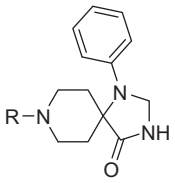

| R | Compound/$EC_{50}$ (μM) | Compound/$EC_{50}$ (μM) |
|---|---|---|
|  | **12a** (**1**)/2.4 | **13a** (**2**)/3.2 |
|  | **12b**/14.8 | **13b**/3.2 |
|  | **12c**/24.9 | – |
|  | **12d**/6.2 | **13c**/4.3 |
|  | **12e**/>50 | **13d**/>50 |
|  | **12f**/>50 | – |
|  | **12g**/>50 | – |
|  | **12h**/>50 | – |

**Table 1** (*continued*)

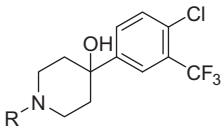
| R | Compound/$EC_{50}$ (μM) | Compound/$EC_{50}$ (μM) |
|---|---|---|
|  | **12i**/>50 | – |
|  | **12j**/4.7 | **13e**/2.7 |
|  | **12k**/2.1 | **13f**/4.5 |

Firstly, changing the linker of the diphenyl ring and alkyl chain (**12b**,[11] **12c**, and **12d**[12]) 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**[13] 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**[14] 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**[15] 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**.

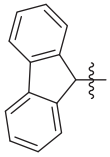
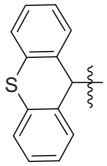
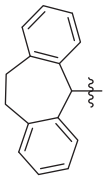
**Table 3**
$EC_{50}$ induction for different tricyclic moieties



**14g-i**



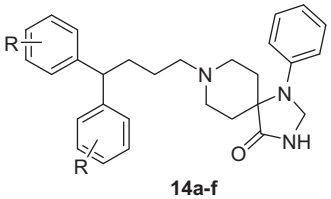
**15k-m**

| R | Compound/$EC_{50}$ (μM) | Compound/$EC_{50}$ (μM) |
|---|---|---|
|  | **14g**/7.9 | **15k**/2.6 |
|  | **14h**/>50 | **15l**/14.4 |
|  | **14i**/5.1 | **15m**/2.6 |

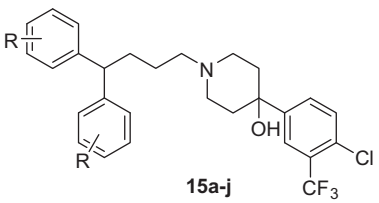
In contrast, one less methylene group gave no change of activity (**12k**),[16] while compound **12f** showed 1.5-fold decreased activity. These results indicated that there may be some tolerance when it comes to the space length (2–4 carbon chain).[17]

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**
$EC_{50}$ induction for diphenyl ring substitution



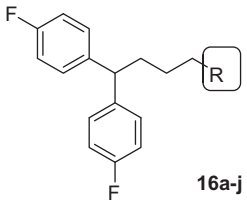
**14a-f**



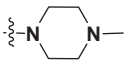
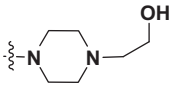
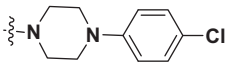
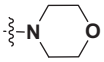
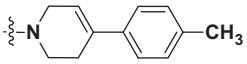
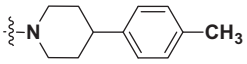
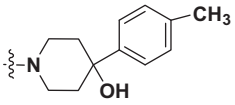
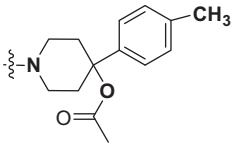
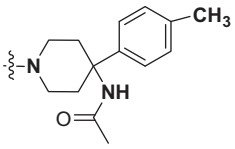
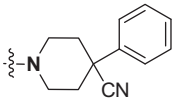
**15a-j**

| R | Compound/$EC_{50}$ (μM) | Compound/$EC_{50}$ (μM) |
|---|---|---|
| H | **14a**/34.6 | **15a**/5.1 |
| 4-F | **14b** (**1**)/2.4 | **15b** (**2**)/3.2 |
| 4-Cl | **14c**/4.2 | **15c**/2.4 |
| 4-$CF_3$ | **14d**/3.7 | **15d**/0.28 |
| 4-$CH_3$ | **14e**/3.1 | **15e**/0.29 |
| 4-$OCH_3$ | **14f**/4.6 | **15f**/1.2 |
| 4-$OCF_3$ | – | **15g**/0.97 |
| 3-F | – | **15h**/2.2 |
| 3-$CF_3$ | – | **15i**/0.65 |
| 3-CH3 | – | **15j**/0.29 |

**Table 4**
$EC_{50}$ induction for right-hand side modification




**16a-j**

| Compound | R | $EC_{50}$ (μM) |
|---|---|---|
| **16a** |  | >50 |
| **16b** |  | >50 |
| **16c** |  | >50 |
| **16d** |  | >50 |
| **16e** |  | >50 |
| **16f** |  | >50 |
| **16g** |  | 6.4 |
| **16h** |  | 4.4 |
| **16i** |  | 2.2 |
| **16j** |  | 10.5 |

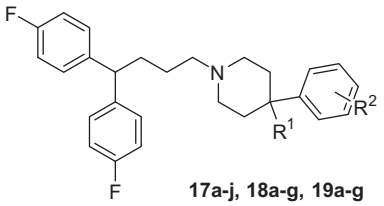
demonstrated the lowest activity in this series. On the other hand, compounds **14c–f** with *para*-substituted diphenyl ring still showed no remarkable improvement in activity compared with compound **14b**. By contrast, compounds **15c–j** with various *para* or *meta*-substituents exhibited promising results. Among them, compound **15d** (R = 4-$CF_3$), **15e** (R = 4-$CH_3$), and **15j** (R = 3-$CH_3$) were ten times more potent when compared with compound **15b**, and compound **15i** (R = 3-$CF_3$) 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.

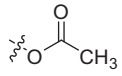
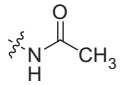
After the modification of the left-hand sides of DPBPs, we decided to examine the effects of the piperidine ring on the bioactivity. As shown in Table 4, compounds **16a–c** with a piperazine ring as well as compound **16d** with a morpholine ring lacked activity completely. Compound **16e**[18] with an unsaturated piperidine and **16f**[18] with no hydroxyl group also showed no inducing activity. As previously reported in the literature,[8] changing of hydroxyl group, either protected by acetyl group (**16h**) or substituted with acetamide group (**16i**), showed good results. However, compounds **16j**[19] 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 $R^1$ to examine their effects on the bioactivity. As shown in Table 5, when $R^1$ = OH, compounds **17b**, **17e–f** with *para*-substituted on the phenyl ring showed better results than compounds **17g–i** with *meta*-substituents, while

**Table 5**
$EC_{50}$ induction for variations at $R^1$ and $R^2$




**17a-j, 18a-g, 19a-g**

| $R^2$ | $R^1$ | | |
|---|---|---|---|
| |  |  |  |
| | Compound/$EC_{50}$ (μM) | Compound/$EC_{50}$ (μM) | Compound/$EC_{50}$ (μM) |
| H | **17a**/12.0 | **18a**/>50 | **19a**/6.2 |
| 4-F | **17b**/6.1 | **18b**/5.4 | **19b**/3.0 |
| 4-Cl | **17c**/7.7 | **18c**/5.0 | **19c**/3.0 |
| 4-Br | **17d**/2.2 | **18d**/2.6 | **19d**/2.5 |
| 4-$CF_3$ | **17e**/4.2 | **18e**/4.5 | **19e**/3.5 |
| 4-$CH_3$ | **17f**/6.4 | **18f**/4.4 | **19f**/2.2 |
| 3-$F_3$ | **17g**/10.1 | — | — |
| 3-$CF_3$ | **17h**/6.7 | — | — |
| 3-$CH_3$ | **17i**/24.6 | — | — |
| 4-Cl-3-$CF_3$ | **17j** (**2**)/3.2 | **18g**/6.3 | **19g**/4.0 |

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,[20] 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 diphenylbutylpiperidines as effective autophagy inducers. An effective synthetic route to diphenylbutyl bromide has also been developed. Structural modifications of compound **1** and **2** bring in significant increase of activity (compounds **15d**, **15e**, **15i**, **15j**). Besides this, effects of structural variations on either the left-hand or the right-hand were studied as well. Moreover, substituents on the piperidine phenyl ring of compound **2** were investigated in the same way. Further SAR exploration to improve the overall biological activity profiles and structural modifications of Pimozide will be reported in due course.

## Acknowledgments

This work was supported in part by grants from the National Natural Science Foundation of China (No. 21072212 to C.Y.) and the National Institute of Health of US (to J.Y.).

## 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

1. (a) Shintani, T.; Klionsky, D. J. *Science* **2004**, *306*, 990; (b) Mizushima, N.; Levine, B.; Cuervo, A. M.; Klionsky, D. J. *Nature* **2008**, *451*, 1069.
2. (a) Lum, J. J.; DeBerardinis, R. J.; Thompson, C. B. *Nat. Rev. Mol. Cell. Biol.* **2005**, *6*, 439; (b) Hara, T.; Nakamura, K.; Matsui, M.; Yamamoto, A.; Nakahara, Y.; Suzuki-Migishima, R.; Yokoyama, M.; Mishima, K.; Saito, I.; Okano, H.; Mizushima, N. *Nature* **2006**, *441*, 885.
3. Zhang, L.; Yu, J.; Pan, H.; Hu, P.; Hao, Y.; Cai, W.; Zhu, H.; Yu, A.; Xie, X.; Ma, D.; Yuan, J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 19023.
4. Williams, A.; Sarkar, S.; Cuddon, P.; Ttofi, E. K.; Saiki, S.; Siddiqi, F. H.; Jahreiss, L.; Fleming, A.; Pask, D.; Goldsmith, P.; O'Kane, C. J.; Floto, R. A.; Rubinsztein, D. C. *Nat. Chem. Biol.* **2008**, *4*, 295.
5. Xia, H.; Zhang, L.; Chen, G.; Zhang, T.; Liu, J.; Jin, M.; Ma, X.; Ma, D.; Yuan, J. *Autophagy* **2010**, *6*, 1.
6. Seeman, P.; Lee, T.; Chau-Wong, M.; Wong, K. *Nature* **1976**, *261*, 717.
7. (a) Gould, R. J.; Murphy, K. M. M.; Reynolds, I. J.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5122; (b) Grantham, C. J.; Main, M. J.; Cannell, M. B. *Br. J. Pharmacol.* **1994**, *111*, 483; (c) Sah, D. W. Y.; Bean, B. P. *Mol. Pharmacol.* **1994**, *45*, 84; (d) Enyeart, J. J.; Biagi, B. A.; Day, R. N.; Sheu, S. S.; Maurer, R. A. *J. Biol. Chem.* **1990**, *265*, 16373; (e) Galizzi, J. P.; Fosset, M.; Romey, G.; Laudron, P.; Lazdunski, M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7513; (f) King, V. F.; Garcia, M. L.; Shevell, J. L.; Slaughter, R. S.; Kaczorowski, G. J. *J. Biol. Chem.* **1989**, *264*, 5633.
8. Hulshof, J. W.; Vischer, H. F.; Verheij, H. F.; Fratantoni, M. H. P.; Smit, M. J.; de Esch, I. J. P.; Leurs, R. *Bioorg. Med. Chem.* **2006**, *14*, 7213.
9. (a) Sindelar, K.; Rajsner, M.; Cervena, I.; Valenta, V.; Jilek, J. O.; Kakac, B.; Holubek, J.; Svatek, E.; Miksik, F.; Protiva, M. *Collect. Czech. Chem. Commun.* **1973**, *38*, 3879; (b) Botteghi, C.; Marchetti, M.; Paganelli, S.; Persi-Paoli, F. *Tetrahedron* **2001**, *57*, 1631; (c) Hulshof, J. W.; Casarosa, P.; Menge, M. P. B.; Kuusisto, L. M.; van der Goot, H.; Smit, M. J.; de Esch, I. J. P.; Leurs, R. *J. Med. Chem.* **2005**, *48*, 6461.
10. Rajsner, M.; Dlabac, A.; Protira, M. *Collect. Czech. Chem. Commun.* **1981**, *46*, 1530.
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.
14. For the synthesis of these left sides of compounds **12f–i**, see Ref.: Janssen, P. A. J.; van der Westeringh, C.; Jageneau, A. H. M.; Demoen, P. J. A.; Hermans, B. K. F.; van Daele, G. H. P.; Schellekens, K. H. L.; van der Eycken, C. A. M.; Niemegeerx, C. J. E. *J. Med. Chem.* **1959**, *1*, 281.
15. The left side of compound **12j** can be synthesized from the same route as showed in Scheme 1 with the starting material δ-valerolactone instead of γ-butyrolactone.
16. Kashali, N.; Polat, M. F.; Altundas, R.; Kara, Y. *Helv. Chim. Acta* **2008**, *91*, 67.
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 **16e** can be prepared from compound **10** (R = $CH_3$) by removing the Boc group with trifluoroacetic acid, and hydrogenation of compound **16e** can give compound **16f**.
19. The right side of compound **16j** is commercially available.
20. Castagnoli, N., Jr.; Rimoldi, J. M.; Bloomquist, J.; Castagnoli, K. P. *Chem. Res. Toxicol.* **1997**, *10*, 924.