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Mendeleev Commun., 2019, **29**, 203–205

Mendeleev Communications

Synthesis and biological evaluation of new N-substituted 4-(arylmethoxy)piperidines as dopamine transporter inhibitors

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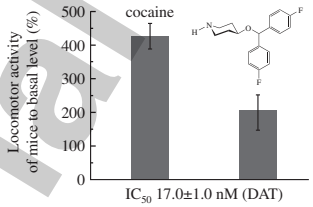
DOI: 10.1016/j.mencom.2019.03.030

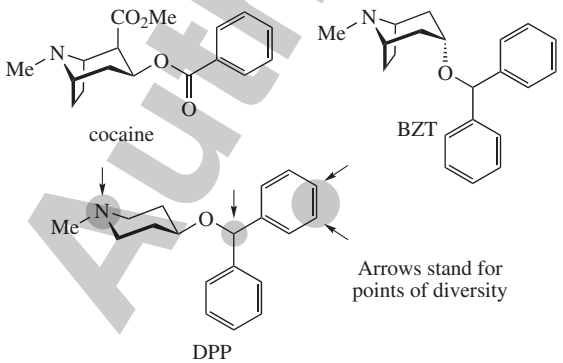
The library of new N-substituted 4-(arylmethoxy)piperidines as dopamine transporter inhibitors was designed and synthesized. H-Bond donors in piperidine ring were found to be important for reduced locomotor activity in mice. 4-[Bis-(4-fluorophenyl)methoxy]piperidine has IC_{50} 17.0±1.0 nM for dopamine transporter and locomotor activity, which is lower than that for cocaine.



The dopamine transporter (DAT) is a transmembrane protein responsible for reuptake of extraneuronal dopamine (DA) in presynaptic terminals of DA neurons, thus being involved in the regulation of dopaminergic transmission.[1–3] DAT has been considered as a primary target of cocaine action.[1,2,4] Development of new DAT inhibitors has been the focus of several publications.[1–4] Such compounds are interesting as molecular tools for the study of DAT structure[1,2] and potential therapeutic agents for treatment of cocaine abuse.[1,2,4] However, selective and potent DAT inhibitors have demonstrated low efficacy for the monotherapy of cocaine addiction.[1,5] Moreover, DAT inhibitors could be promising for treatment of some 'dopamine diseases', *i.e.,* attention deficit and hyperactivity disorders as well as Parkinson's disease.[6] Recently DAT inhibitors attracted persistent attention as components for triple reuptake inhibitors for antidepressant development.[3] Enhanced studies of DAT inhibitors with piperidine ring revealed its influence on H-1 and H-3 central histamine receptors.[7–10] This influence appears as an antagonism to the behavioural and locomotor activity in rodent models of behavioural disorders.[7–10] Throughout the past decade, benzotropine (BZT) analogues have been shown to be potent DAT inhibitors.[7] While the DAT binding affinities of BZT analogues are superior to cocaine, their locomotor activities are less due to affinity to the several M- and H-receptors.[7] A recent study examined the binding of BZT analogues to H-1, H-2, and H-3 histamine receptors and showed that BZT analogues have modest central antihistamine effects.[11] It serves only as a first step towards understanding integral activity of BZT analogues on behavioural reactions such as locomotor activity.[11] Some of histamine antagonists comprised piperidin-4-ol fragment, a popular scaffold for development of H-1-[12] and H-3-hystamine receptor antagonists.[13]

4-(Diphenylmethoxy)piperidine (DPP) **1** is a well-known H-1-histamine antagonist with strong DAT inhibiting activity.[14,15] It is known that alpha-enantiomer of BZT is much more active than beta-one in binding analysis.[4] 3D-structure of alpha-BZT does not completely correspond to that of cocaine since there is agreement only in tropane cycle but position of benzene moiety in the space is different. Meanwhile, DPP **1** has conformationally mobile piperidine cycle lacking two-carbon bridge. Thus, there is no limitation in configuration at C-4 of the piperidine cycle. Piperidine cycle, ether bond and one aromatic ring can coincide in space, which is important for cocaine pharmacophore.[2,4] The alignment of molecules was made along the piperidine cycle and ether bond (Figure 1).[16] The root-mean-square deviation (RMSD) value for this alignment was 3.28 that was acceptable for the proposals of pharmacophore similarity and acceptance of qualitative speculations about side radicals for this core.[16,17]

Previously, we discussed the comparison of pharmacological profiles for cocaine and DPP and the benefits of 4-fluorophenyl substituents in diarylmethoxy fragment.[14,15] Also, we described simple approach to the combinatorial library and more potent DAT inhibitors with diverse locomotor activity *in vivo*.[15] This approach involved combinatorial modification of three points of diversity in DPP structure and was limited by the nitrogen of piperidine ring, methine group of diarylmethoxy fragment and aromatic rings.[15]



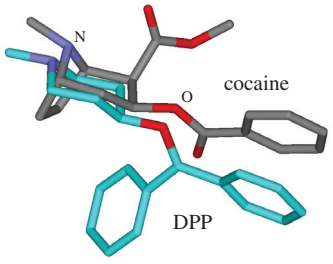


Figure 1 The alignment of DPP along the cocaine structure.

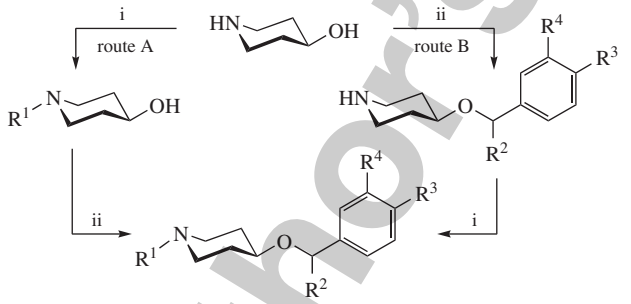
Table 1 Biological evaluation and calculated log*P* data for compounds **1–15** and cocaine.

Compound	Experimental IC_{50}/nM DAT, ^{123}I-RTI-55	Experimental K_i/nM SERT, ^{3}H-citalopram	Motion activity to basal level 17.8 mmol kg^{-1} (%)	Calc. log*P*
1 (DPP)	420 ± 9.0[a]	13587 ± 1920	311.0[a]	3.2
2	22.1 ± 5.7[a]	7518 ± 1690	887.8[a]	3.5
3	12.5 ± 7.5[a]	396 ± 57	98.7[a]	6.0
4	44.0 ± 10.9[a]	> 14000	150.2[a]	4.5
5	50.6 ± 2.8[a]	406 ± 100	41.4	6.1
6	155 ± 10.0[a]	2426 ± 130	159.4[a]	3.8
7	264 ± 9.0[a]	> 14000	28.9	2.1
8	277 ± 10.0[a]	3083 ± 330	126.5	3.3
9	293 ± 10.0[a]	5534 ± 1720	88.8	3.6
10	32.0 ± 1.4	> 14000	182.0	4.9
11	61.6 ± 7.2	> 14000	140.5	4.2
12	5353 ± 160	> 14000	91.7	4.4
13	6000 ± 150	> 14000	99.3	3.6
14	17 ± 2.0	> 14000	206.7	2.9
15	251 ± 12.0	> 14000	8.5	3.3
Cocaine	104 ± 49.0[a]	297 ± 13.0	426.0[a]	2.9

[a] See refs. 14 and 15.

Here we describe the further improvement and optimization of the leading compound efficacy as well as the synthesis and biological evaluation of novel 4-(4,4′-difluorobenzhydryloxy)-piperidines and compare them with prototypical DAT inhibitors such as early described compounds **1** and **2** (Table 1). Additionally we provide new data on binding properties of earlier synthesized compounds **1–9** and biological evaluation for the new ones **10–15**.

N-Methyl-4-(4,4′-difluorobenzhydryloxy)piperidine **2** was chosen as a leading compound to increase DAT affinity because it showed high affinity and selectivity to DAT. This compound has shown remarkably increased locomotor activity which depends on the penetration through blood–brain barrier.[15] We used big–small and donor–acceptor pairs of substituents for the new compounds to expand previously described combinatorial library since our alignment showed some similarity to the cocaine structure and some similarity to the BZT analogues. From this point of view, new six compounds **10–15** (see Table 1) were chosen for the synthesis. *p*-Fluoro substituents in diarylmethoxy fragment can provide two symmetrical H-bond acceptors. *N*-Butyl or *N*-isopropyl are bulky substituents which can increase activity in comparison to compound **2**. (*p*-Tolyl)methoxy fragment can account for an importance of *para*-substituent with donor properties. Additionally, 3,4-dichlorophenyl fragment could present the series of *meta*-substituents. The influence of size of *para*-substituents can explain small (fluoro-) or large (chloro-) side groupings. *N*-Cyanomethyl fragment can provide knowledge on the effect of donor–acceptor type of interaction.



1–15

	R^1	R^2	R^3	R^4		R^1	R^2	R^3	R^4
1	Me	Ph	H	H	**9**	Me	Ph	H	Me
2	Me	4-FC_6H_4	F	H	**10**	Bu	4-FC_6H_4	F	H
3	Bu	4-ClC_6H_4	Cl	H	**11**	Pr^i	4-FC_6H_4	F	H
4	Me	4-ClC_6H_4	Cl	H	**12**	Me	Ph	Cl	Cl
5	4-$FC_6H_4CH_2$	4-ClC_6H_4	Cl	H	**13**	Me	Ph	Me	H
6	Me	Ph	Cl	H	**14**	H	4-FC_6H_4	F	H
7	Me	Me	F	H	**15**	CH_2CN	4-FC_6H_4	F	H
8	Me	Ph	F	H					

Scheme 1 *Reagents and conditions:* i, R^1Hal, K_2CO_3, DMF, 40 °C; ii, 3-R^4-4-R^3-$C_6H_3CH(R^2)OH$, TsOH, toluene–DMF (25 : 1), reflux.

We used 4-(benzhydryloxy)piperidins as the scaffold with three points of diversity for N–H group, OCH fragment and aromatic rings. Here we describe substitution at the N-atom and in aromatic rings (Scheme 1). Recently,[15] we reported on route A. Obviously, route B is more economical and suitable for combinatorial synthesis to introduce substituents at the nitrogen atom of piperidine moiety at the last step and it was used to prepare new compounds **10–15**.†

Commercially available substituted benzophenones were reduced ($NaBH_4$, Pr^{i}OH) to benzhydrols in nearly 100% yield. The etherification of the latter with 5% molar excess of piperidin-4-ols or *N*-methylpiperidin-4-ol was performed by reflux in toluene with a Dean–Stark trap in the presence of TsOH,[15,18] the yields of the ethers ranged from 60 to 90%. N-Substituted piperidines **10**, **11**, **15** were prepared by alkylation of 4-[bis(4-fluorophenyl)-methoxy]piperidine with alkyl halides in DMF in the presence of anhydrous potassium carbonate in yields of approximately 90%. Some compounds were converted into salts with oxalic acid in acetone, which improved their solubility in water. Table 1 contains the structures, data of binding assay and locomotor activity of compounds.

Binding assay was performed as described previously.[19] Determination of doses for *in vivo* tests (17.8 mmol kg^{-1}) and locomotor activity test was reported in previous publications.[14,15] The results of locomotor activity test were expressed as percents to the basal level of motion activity when mice were injected with saline (Figure 2). Synthesized compounds significantly changed locomotor activity levels ($P < 0.05$). Statistical one-way analysis of variance (ANOVA) was carried out.

Our IC_{50} results have revealed the same trend of SAR known from published series of BZT analogues.[2–4,7] The use of our scaffold (DPP or **3**) with lower molecular weight as compared to BZT allowed us to avoid some limits of 'Lipinsky's rule of five'. Using known method[20] we calculated log*P*, which helped us to obtain compounds with better balance, lower molecular weight and lipophilicity by increasing size of substituents at the N atom of piperidine ring.[20] Binding assay showed that all compounds have excellent selectivity to DAT in comparison to SERT (see Table 1). Motion activity tests revealed less predictable results than IC_{50} because it reflects total effects of DAT inhibitors in living animal. This trend depends on factors such as binding

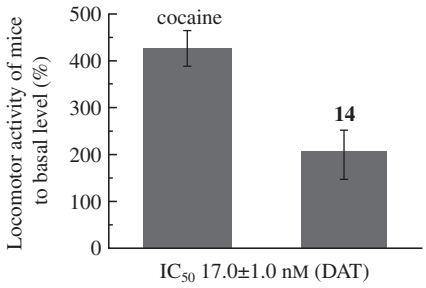


Figure 2 Comparison of locomotor activity in mice for cocaine and compound **14** in doses 17.8 mmol kg^{-1} given as a percentage to basal level (injection of saline).

† For the synthetic details, see Online Supplementary Materials.

affinity, penetration through blood–brain barrier and distribution among different types of receptors.

Almost all compounds had IC_{50} better than that of DPP. As we predicted and found earlier,[15] the introduction of lipophilic moieties at nitrogen atom in compounds **2**, **5**, **10** led to an increase in IC_{50}, however increase in *N*-alkyl size was not sufficient. Although, the structure of compound **7** has the best alignment with the cocaine one (see Scheme 1) because carbonyl group was changed with methyl one, this did not lead to an increase in IC_{50}. The most active compound **3** had the strongest IC_{50} and biggest log*P* but it is too lipophilic to penetrate through the blood brain barrier (see Table 1).[15] Both H-bond donors at piperidine nitrogen (compound **14**) and alkyl substituents (compounds **2**, **10** and **11**) caused an increase in IC_{50}. However, the *in vivo* tests have shown a lot of restrictions for this direction in piperidine scaffold modification.

Almost all compounds have shown decrease in motion activity test as compared to cocaine. For compounds DPP, **6**–**9**, **13** it could depend on smaller affinity in comparison with that of cocaine. Reduced locomotor activity of mice for compounds **2**–**5**, **10**, **11** could be due to insufficient penetration through the blood–brain barrier and distribution among fat tissue because log *P* values for these compounds were too high. IC_{50} values of compounds **7** and **15** are low or moderate but changes of *in vivo* effects are sharp. Probably, effect of **7** and **15** results from mechanism of motion activity regulation differing from those described and discussed previously.[14,15] Compounds **14** and **15** had reduced locomotor activity, which could be due to changing in interaction balance with receptors since these compounds bear hydrogen bond donors at N-site of piperidine fragment. Two explanations of these results can be proposed on the basis of aforementioned information. First, compound **15** has good agreement with the 'rule of five', therefore it should have good distribution and penetration. Also, its electron-rich cyano group at N-substituent can seriously influence the affinity to histamine receptor and can reduce motion activity. To this, N-substituted 4-(diphenylmethoxy)piperidines are known to have antihistamine properties,[12,16] and the central effects of this action would reduce motion activity. Further tests of binding new compounds to the central H-1 and H-3 histamine receptors seem interesting.

In conclusion, we revealed that DPP analogues with lipophilic N-substituents as well as ones *para*-substituted in benzhydroxy fragment showed the trend of increasing the IC_{50} value. Both aliphatic and halogen *meta*-substituents in benzhydryloxy moiety cause decrease in IC_{50}. The most promising candidates for further studies are new compounds **11**, **14** and **15**. Perhaps, the introduction of H-bond acceptors into *N*-piperidine substituent can be a good tool for further improving activity within this combinatorial library.

We are grateful to Professors S. R. Jones and S. R. Childers (Wake Forest University Health Science, NC) and Dr. Jill J. Harp (Department of Life Sciences, Winston-Salem State University, NC) for comprehensive consultation on binding experiments, lab routines of radioactive safety and *in vivo* experiments. We are thankful to Dr. V. Grinevich (Asinex, NC) for friendly advice and comments.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2019.03.030.

References

1 B. Gryzło, P. Zaręba, K. Malawska, A. Jakubowska and K. Kulig, *Curr. Med. Chem.*, 2015, **22**, 3255.
2 S. Singh, *Chem. Rev.*, 2000, **100**, 925.
3 G. B. Lapa, *Pharm. Chem. J.*, 2011, **45**, 323.
4 A. K. Dutta, S. Zhang, R. Kolhatkar and M. E. A. Reith, *Eur. J. Pharmacol.*, 2003, **479**, 93.
5 D. C. Roberts, *Physiol. Behav.*, 2005, **86**, 18.
6 P. Huot, S. H. Fox and J. M. Brotchie, *J. Pharmacol. Exp. Ther.*, 2016, **357**, 562.
7 R. I. Desai, T. A. Kopajtic, D. French, A. H. Newman and J. L. Katz, *J. Pharmacol. Exp. Ther.*, 2005, **315**, 397.
8 A. Blokland, B. Scholtissen, A. Vermeeren and J. Ramaekers, *Pharmacol. Biochem. Behav.*, 2001, **70**, 427.
9 T. E. Wilens, *Drugs*, 2003, **63**, 2395.
10 C. A. Dvorak, R. Apodaca, A. J. Barbier, C.W. Berridge, S. J. Wilson, J. D. Boggs, W. Xiao, T. W. Lovenberg and N. I. Carruthers, *J. Med. Chem.*, 2005, **48**, 2229.
11 V. C. Campbell, T. A. Kopajtic, A. H. Newman and J. L. Katz, *J. Pharmacol. Exp. Ther.*, 2005, **315**, 631.
12 A. H. Newman, S. Izenwasser, M. J. Robarge and R. H. Kline, *J. Med. Chem.*, 1999, **42**, 3502.
13 P. A. Petukhov, J. Zhang, C. Z. Wang, Y. P. Ye, K. M. Johnson and A. P. Kozikowski, *J. Med. Chem.*, 2004, **47**, 3009.
14 G. B. Lapa, T. A. Mathews, J. J. Harp, E. A. Budygin and S. R. Jones, *Eur. J. Pharmacol.*, 2005, **506**, 237.
15 G. B. Lapa, G. B. Byrd, A. A. Lapa, E. A. Budygin, S. R. Childers, S. R. Jones and J. J. Harp, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4915.
16 S. Putta and P. Beroza, *Curr. Top. Med. Chem.*, 2007, **7**, 1514.
17 C. Lemmen and T. J. Lengauer, *Comput. Aided Mol. Des.*, 1997, **11**, 357.
18 *Patent GB 702067*, 1954 (*Chem. Abstr.*, 1955, 4023i).
19 S. R. Letchworth, H. R. Smith, L. J. Porrino, B. A. Bennett, H. M. L. Davies, T. Sexton and S. R. Childers, *J. Pharmacol. Exp. Ther.*, 2000, **293**, 686.
20 P. Ertl, B. Rohde and P. Selzer, *J. Med. Chem.*, 2000, **43**, 3714.

Received: 29th June 2018; Com. 18/5623