Bioorganic & Medicinal Chemistry 21 (2013) 676–683

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and evaluation of novel heterodimers of donepezil and huperzine fragments as acetylcholinesterase inhibitors

Yueqing Hu [a,c], Jun Zhang [a,c], Oormila Chandrashankra [a,c], Fanny C. F. Ip [a,b,c], Nancy Y. Ip [a,b,c,*]

[a] Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China
[b] State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China
[c] Biotechnology Research Institute, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

ARTICLE INFO

Article history:
Received 12 October 2012
Revised 26 November 2012
Accepted 27 November 2012
Available online 6 December 2012

Keywords:
Alzheimer's disease
Acetylcholinesterase
Heterodimer
Huperzine A

ABSTRACT

Four series of novel heterodimers comprised of donepezil and huperzine A (HupA) fragments were designed, synthesized, and evaluated in search of potent acetylcholinesterase (AChE) inhibitors as potential therapeutic treatment for Alzheimer's disease. Heterodimers comprised of dimethoxyindanone (from donepezil), hupyridone (from HupA), and connected with a multimethylene linker, were identified as potent and selective inhibitors of AChE. Diastereomeric heterodimers (***RS*,*S*)-17b** (with a tetramethylene linker) exhibited the highest potency of inhibition towards AChE with an IC_{50} value of 9 nM and no detectable inhibitory effect on butyrylcholinesterase at 1 mM.

1. Introduction

Alzheimer's disease (AD) is a common age-related neurodegenerative disorder characterized by progressive cognitive dysfunction and memory loss, neuropsychiatric and behavioral disturbances, and restrictions in daily activities.[1] Recent report indicates that approximately 36 million people worldwide suffer from AD, and this number is projected to reach 115 million by the year 2050.[2] Although tremendous progress has been made in understanding the pathophysiological mechanisms underlying AD, at present there is no cure for this disease. The current medications used for the symptomatic treatment of AD mainly belong to a class of acetylcholinesterase (AChE) inhibitors which enhance central cholinergic neurotransmission by inhibiting the degradation of acetylcholine.[3] Figure 1 depicts the structures of current therapeutic agents for AD. Out of the five medications on the market, four of them (tacrine, donepezil, rivastigmine and galantamine) are AChE inhibitors, while memantine is a NMDA receptor antagonist. However, all of these medicines show unwanted side effects in some of the patients and their effectiveness diminishes after prolonged treatment.[4] Due to the rapidly increasing aging population globally, there is a huge demand for unmet medical need.

The search for novel AChE inhibitors with improved biological profiles continues to be of great interest to medicinal chemists. Hybridization and dimerization of two structural units of one or two lead compounds of interest have proven to be successful strategies in the discovery of novel AChE inhibitors. Dimeric AChE inhibitors exhibit improved pharmacological profiles as they can interact with both the active and peripheral sites of AChE, or simultaneously interact with AChE and another biological target involved in Alzheimer's disease.[5] Since the discovery of tacrine homodimers in the mid-1990s,[6] a variety of dimeric AChE inhibitors have been identified over the years.[7–15] For example, homodimers of two inactive hupyridones from huperzine A (HupA), heterodimers of hupyridone and tacrine, and heterodimers of donepezil and tacrine, have been reported to be potent AChE inhibitors.[7–11]

We report here our efforts in search of novel potent AChE inhibitors using the dimerization strategy. A number of heterodimers, with structural moieties derived from two known AChE inhibitors, donepezil and HupA, were designed, synthesized, and evaluated.

2. Results and discussion

2.1. Design and chemistry

The heterodimers were designed based on the structures of two potent AChE inhibitors, donepezil and HupA (Fig. 2). The binding modes of donepezil and HupA with AChE have been revealed by 3D X-ray structures, molecular docking, and mutagenesis studies.[16,17] Donepezil interacts with AChE at both the active-site gorge and the peripheral anionic site. While the dimethoxyindanone moiety of donepezil binds to the peripheral site, the piperidine

* Corresponding author. Tel.: +852 2358 7267; fax: +852 2358 1552.
E-mail address: boip@ust.hk (N.Y. Ip).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.11.044

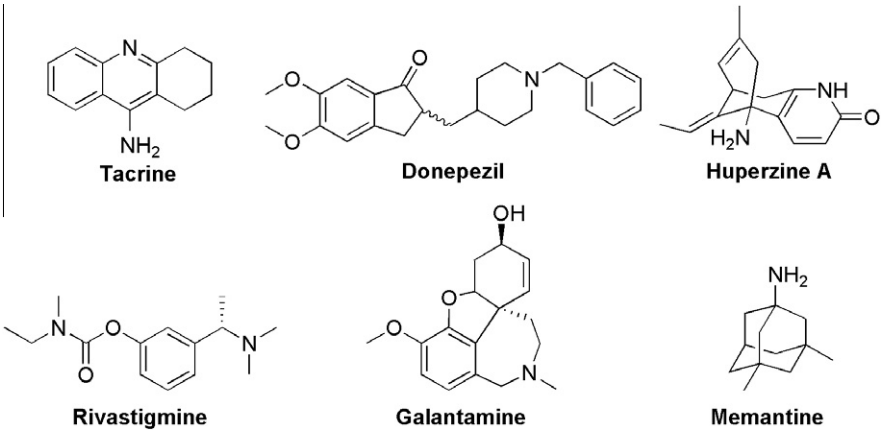


Figure 1. Current therapeutic agents for AD.

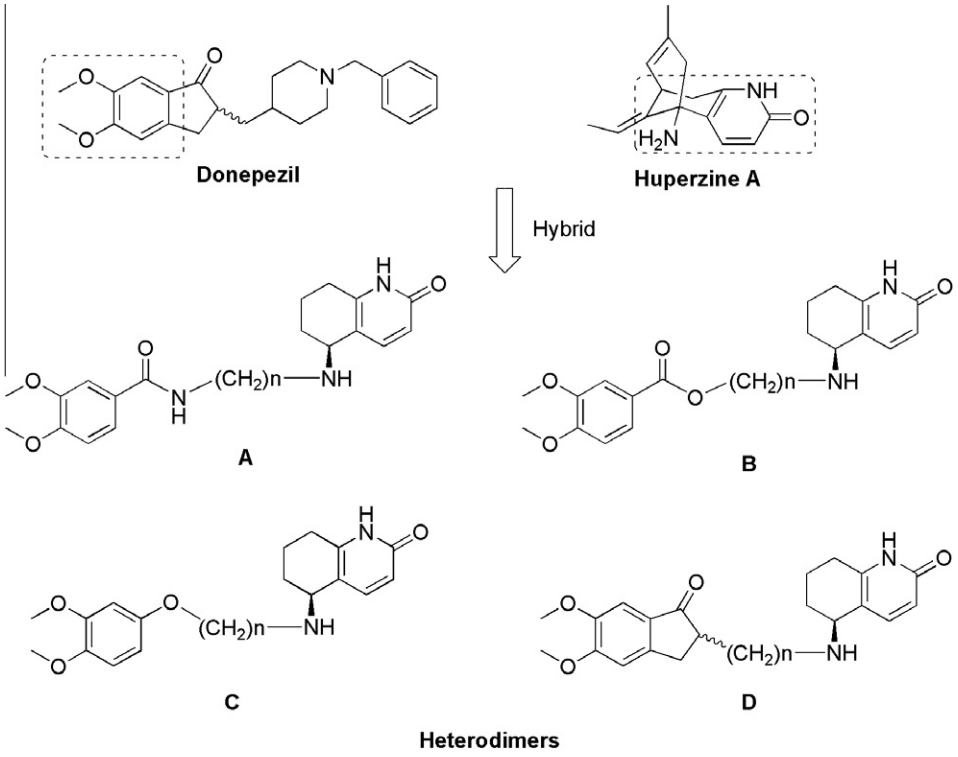
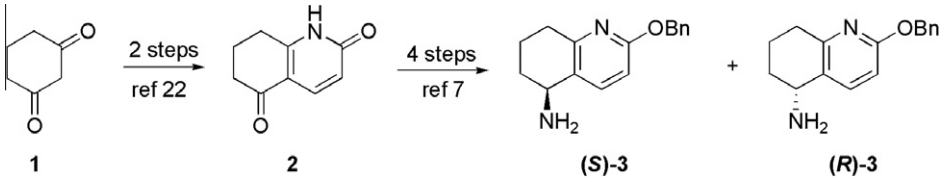


Figure 2. Design strategy for heterodimers of donepezil and huperzine A fragments.

and benzyl moieties bind to the mid and lower gorge, the active site of AChE. Donepezil does not bind to the catalytic triad.[18] HupA, however, binds to the AChE active-site gorge and the catalytic triad but not the peripheral site, either directly or indirectly via the carbonyl oxygen, the ring amine, the 5-amino and the ethylidene methyl groups.[19]

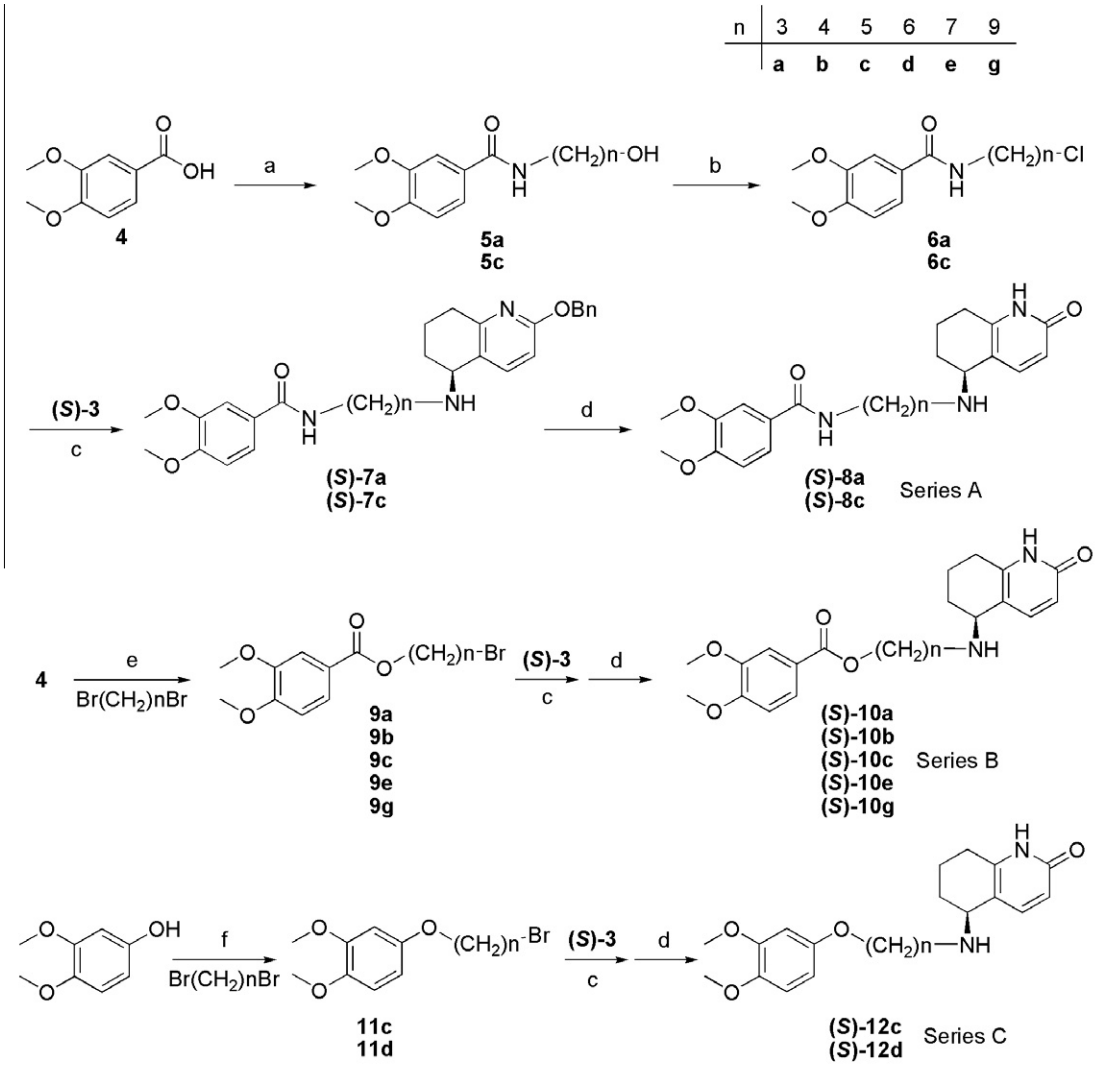
In view of the superior potency of donepezil and HupA, a total of four series of heterodimers were designed. These heterodimers consisted of a dimethoxybenzene unit from donepezil and a hupyridone fragment from HupA linked by a chain of methylenes. Based on the crystallographic findings on the complexes comprised of *Torpedo californica* AChE (*Tc*AChE) with either donepezil, HupA or bishupyridones,[18–20] it was speculated that the dimethoxybenzene unit would interact with the peripheral site while the hupyridone fragment would bind to the active site of the AChE. The hupyridone fragment could act as a bioisosteric structure of benzylpiperidine as both fragments contain basic nitrogen and aromatic units. Since the carbonyl oxygen of donepezil was involved in AChE binding, an oxygen atom at a similar position was preserved in the heterodimer structures. In series A, an amide bond was used to connect the dimethoxyphenyl to the multimethylene linker whereas in series B and C, an ester and ether bond were put in place, respectively. In series D, the dimethoxyindanone ring was preserved, and was directly connected to the multimethylene linker. Since the number of carbons between the carbonyl and basic nitrogen in donepezil is 5, heterodimers with a linker consisting of 3–9 methylenes were synthesized and evaluated. Heterodimers of series A, B, and C were enantiomerically pure, while compounds of series D were expected to be mixtures comprising of a pair of diastereomers due to the racemic nature of the C_2 stereogenic center of the indanone ring. The configuration of C_2 indanone system in series D was expected to interconvert via a keto-enol intermediate based on studies of donepezil.[21]



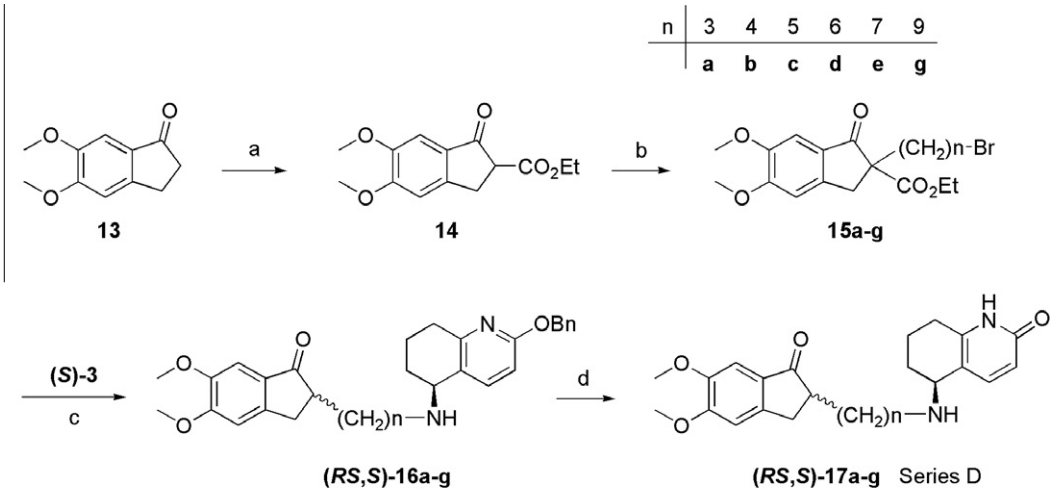
Scheme 1. Synthesis of hupyridone building blocks.

The benzyl protected hupyridone building block (***S***)-**3** and its enantiomer **(*R*)-3** were synthesized from a readily available starting material—dihydroresorcinol **1** through intermediate **2** in 6 steps according to literature (Scheme 1).[7,22] The enantiomeric excess of **(*S*)-3** and **(*R*)-3** were both >97%, as determined by optical rotation analysis.

Synthesis of heterodimers with A, B, and C scaffolds was accomplished as shown in Scheme 2. The *S*-enantiomer of hupyridone benzyl ether **3** was utilized to prepare these heterodimers. The starting material for both series A and B was 3,4-dimethoxybenzoic acid **4**. Treatment of acid **4** with thionyl chloride generated 3,4-dimethoxybenzoyl chloride, which was then reacted with α,ω-multimethylene hydroxylamine to produce amides **5a/5c**. The terminal hydroxyl groups of **5a/5c** were then converted to the corresponding chlorides with thionyl chloride to generate compounds **6a/6c**. Mixing of the chlorides **6a/6c** with amine **(*S*)-3** without addition of any base, and subsequently heating the mixture to 130 °C yielded heterodimers **(*S*)-7a/7c**. Deprotection of the benzyl ether by catalytic hydrogenation generated the target heterodimers **(*S*)-8a/8c** of series A. Treatment of **4** with α,ω-dibromoalkane in the presence of potassium carbonate generated bromides **9a–g**. Reaction of bromides **9a–g** with amine **(*S*)-3**, followed by deprotection of the benzyl group resulted in heterodimers **(*S*)-10a–g** of series B. For series C, combination of the dimethoxyphenol with



Scheme 2. Synthesis of heterodimers A, B and C series.



Scheme 3. Synthesis of heterodimers of D series.

α,ω-dibromoalkanes in the presence of sodium hydride produced bromides **11c/11d**. Target products **(*S*)-12c/12d** were then obtained by reacting the bromides **11c/11d** with amine **(*S*)-3** followed by benzyl deprotection.

The heterodimers of series D were prepared as shown in Scheme 3. Synthesis was initiated by treating dimethoxyindanone **13** with sodium hydride and diethyl carbonate to incorporate an ethoxycarbonyl group at the α-position of the carbonyl thus generating **14**. Mono-alkylation of **14** with α,ω-dibromoalkanes in the presence of sodium hydride provided **15a–g** with a terminal bromide. Diastereomeric mixtures (***RS*,*S*)-16a–g** were then prepared by reacting racemic bromides **15a–g** with chiral amine **(*S*)-3** by heating toluene to form heterodimers with the carboxyl ethyl ester attached, followed by saponification with sodium hydroxide and decarboxylation to remove the carboxyl ethyl ester. Catalytic hydrogenation removed the benzyl protecting group in **(*RS*,*S*)-16a–g**, thus generating the desired heterodimers **(*RS*,*S*)-17a–g**. Alternatively, another pair of diastereomers (***RS*,*R*)-17b** were synthesized from racemic intermediate **15b** ($n = 4$) and chiral amine (***R*)-3** following the same methodology.

Although the heterodimers of series D are a pair of diastereomers due to the racemic nature of the C_2 position on the indanone ring, there were no detectable differences in ^{1}H NMR and ^{13}C NMR of the heterodimers with a linker comprised of more than 5 methylenes. For diastereomers (***RS*,*S*)-17a** and (***RS*,*S*)-17b** that consisted of shorter linkers (3 or 4 methylenes), duplicated doublets were detected in the ^{1}H NMR at ~7.45 ppm and in a ratio of 1:1. The configuration of the stereogenic center at C_2 position of the indanone ring could interconvert via a keto–enol intermediate as observed for donepezil,[21] but no effort was made to separate the two diastereomers in this study.

2.2. Biological activity

The AChE and BuChE inhibitory activities of the heterodimers were determined by a modified version of the Ellman's method.[23] AChE and BuChE were freshly prepared from the cortices and serum of adult rats, respectively. At least three independent experiments were performed to evaluate the efficacies of the heterodimers on cholinesterase inhibition.

The data is summarized in Table 1. Serving as controls, donepezil hydrochloride, HupA, and tacrine exhibited inhibitory effects against AChE with IC_{50} of 32, 65, and 249 nM, respectively. The IC_{50} and selectivity of AChE over BuChE for donepezil, HupA, and tacrine resulting from the assay were within the range reported in literature.[7,9,18,19] Across the different series—series A with the amido-multimethylene linker, series B with the carboxy-multimethylene linker, and series C with the oxy-multimethylene linker—all of the compounds exhibited poor AChE inhibition potency with IC_{50} in the micromolar range. They were much less potent than either of the two original compounds. Of the three series, compound **(*S*)-12c** of series C, consisting of a pentamethylene linker, exhibited the best activity towards AChE inhibition with an IC_{50} of 590 nM and an affinity for AChE 70 times greater than for BuChE. Importantly, a tether length-dependent AChE potency was observed in series B. Compound **(*S*)-10b**, which contained 4 methylenes, most effectively inhibited AChE within the series, indirectly indicating a dual binding mode for these compounds.

Interestingly, compounds that exhibited the most promising inhibitory effect on AChE belonged to series D, which were heterodimers of dimethoxyindanone and hupyridone. Diastereomeric mixture (***RS*,*S*)-17b** with a linker of 4 methylenes showed the strongest inhibition towards AChE with an IC_{50} of 9 nM. It was equally potent to donepezil (reported $IC_{50} = 5.7$ nM)[24], and ~7-fold and ~28-fold more potent towards AChE inhibition than HupA and tacrine. Diastereomers **(*RS*,*S*)-17a** and (***RS*,*S*)-17c** with only one methylene different from (***RS*,*S*)-17b** were ~4600- (41 μM) and ~20-fold (187 nM) less potent than (***RS*,*S*)-17b** towards AChE inhibition, indicating the importance of the length of the linker in AChE inhibition potency. Furthermore, the heterodimers in series D, especially **(*RS*,*S*)-17a–d**, exhibited high selectivity for AChE as they did not show any inhibitory effect on BuChE at the maximum concentration tested (1 mM). In particular, (***RS*,*S*)-17b** exhibited greater than 100,000-fold selectivity for AChE inhibition (assuming the IC_{50} for BuChE was ~1 mM), which was a substantial increase in specificity when compared with donepezil, Hup A, and tacrine (1344, 1138, and 0.6, Table 1).

The dimethoxyindanone unit of donepezil binds to Trp279 located at the peripheral site of *Tc*AChE. Due to the lack of a corresponding aromatic residue in BuChE, the binding of dimethoxyindanone unit to the indole ring of Trp279 accounts for the higher selectivity on AChE of donepezil.[19] The presence of a dimethoxyindanone unit in our heterodimers might preserve this advantage on the selectivity. In accordance with Cardozo's report,[25] the carbonyl group of the indanone ring of donepezil is important for its higher potency in AChE inhibition, since our series

Table 1
In vitro inhibitory activities of heterodimers against AChE and BuChE

Compound[a]	Series	*n*	AChE IC_{50} (nM)	BuChE IC_{50} (nM)	BuChE IC_{50}/AChE IC_{50}
(*S*)-8a	A	3	88,000 ± 10,000	139,000 ± 16,000	1.6
(*S*)-8c	A	5	12,000 ± 1800	33,000 ± 11,000	2.7
(*S*)-10a	B	3	162,000 ± 46,000	860,000 ± 160,000	5.3
(*S*)-10b	B	4	4100 ± 2000	15,000 ± 1000	3.7
(*S*)-10c	B	5	7200 ± 700	460,000 ± 57,000	64
(*S*)-10e	B	7	8200 ± 1000	194,000 ± 67,000	24
(*S*)-10g	B	9	9670 ± 30	308,000 ± 48,000	32
(*S*)-12c	C	5	590 ± 10	42,000 ± 2000	71
(*S*)-12d	C	6	3950 ± 60	17,700 ± 9700	4.5
(*RS*,*S*)-17a	D	3	41,000 ± 3000	—[b]	ND[c]
(*RS*,*S*)-17b	D	4	9 ± 1	—	ND
(*RS*,*S*)-17c	D	5	187 ± 33	—	ND
(*RS*,*S*)-17d	D	6	37 ± 8	—	ND
(*RS*,*S*)-17e	D	7	1860 ± 40	413,000 ± 49,000	222
(*RS*,*S*)-17g	D	9	2600 ± 40	678,000 ± 52,000	261
(*RS*,*R*)-17b	D	4	530 ± 120	—	ND
Donepezil			32 ± 16	43,000 ± 7200	1344
(−)-Huperzine A			65	74,000	1138
Tacrine			249 ± 19	137 ± 19	0.6

[a] All of the compounds were assayed as their HCl salts.
[b] No inhibition at 1 mM.
[c] Not detectable.

A–C lacking an intact indanone ring showed poorer inhibitory effect on AChE when compared to series D. Furthermore, when comparing (***RS*,*S***)**-17b** with (***RS*,*R***)**-17b**, (***RS*,*R***)**-17b** inhibited AChE ~60-fold less potently with an IC_{50} of 530 nM. The observations for (***RS*,*S***)**-17b** and (***RS*,*R***)**-17b** were also seen in the homodimer of hupyridones. The (*S*,*S*)-enantiomer exhibited a ~60-fold greater potency than its (*R*,*R*)-form.[7]

Our data further supports the notion that the absolute configuration of the stereogenic center near the basic nitrogen greatly affects the potency of the AChE inhibitor.[7] The optimal tether length between indanone and hupyridone of heterodimer D series is 4 methylenes. In the structure of donepezil, the space between the indanone ring and basic nitrogen is also four carbon units. These results indicate that our design strategy of replacing *N*-benzylpiperidine with the bioisosteric hupyridone moiety successfully resulted in generating potent and selective AChE inhibitors. However, since the heterodimers in series D are a pair of diastereomers, data analysis for pharmacological potency of these compounds should be taken with caution. It will be of further interest to examine their individual potency towards cholinesterase inhibition.

3. Conclusion

In conclusion, based on the superior binding properties of donepezil and HupA with AChE,[18–20] we have designed and synthesized four different series of heterodimers consisting of donepezil and HupA fragments, and have evaluated their effectiveness as anti-cholinesterase agents. Potent and selective AChE inhibitors were identified from novel heterodimers comprising of dimethoxyindanone from donepezil and hupyridone from HupA (series D). Heterodimers (***RS*,*S***)**-17b** linked by 4 methylenes exhibited the highest potency of inhibition towards AChE with an IC_{50} value of 9 nM and no detectable inhibitory effect on BuChE at 1 mM.

4. Experimental section

4.1. Chemistry

All reagents and starting materials were purchased from commercial sources and used without further purification. ^{1}H NMR and ^{13}C NMR were recorded using a Varian 300 MHz or Joel 400 MHz spectrometer. Chemical shifts of ^{1}H NMR spectra were measured in parts per million referenced to residual solvent (chloroform at 7.26, TMS at 0.00, methanol at 3.30 ppm). Coupling constants were measured in hertz. Chemical shifts of ^{13}C NMR were referenced to residual solvent (chloroform at 77.0, methanol at 49.0 ppm). HRMS (ESI) was obtained using API QSTAR XL Mass Spectrometer system. Optical rotations were measured using a JASCO P-2000 digital polarimeter. Final compounds in HCl salt form were used for measuring the optical rotation.

4.1.1. 5,6–Dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester (14)

To a solution of diethyl carbonate (1.89 mL, 15.6 mmol), NaH (0.46 g, 15.6 mmol, 80% dispersion in mineral oil) in toluene (10 mL), a solution of 5,6-dimethoxy-indan-1-one **13** (1.50 g, 7.8 mmol) in toluene (20 mL) was added dropwise over a 1 h period, and the mixture was stirred at reflux for 5 h. After cooling to room temperature, the reaction was quenched with water (30 mL) and acetic acid (1.5 mL). The resulting mixture was extracted with dichloromethane (3 × 30 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Purification by flash column chromatography (silica gel, EtOAc/PE, 3/7) generated the title compound (1.76 g, 85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18 (s, 1H), 6.92 (s, 1H), 4.24 (q, J = 7.0 Hz, 2H), 3.98 (s, 3H), 3.91 (s, 3H), 3.69–3.72 (m, 1H), 3.45 (dd, J = 17.0, 3.5 Hz, 1H), 3.28 (dd, J = 17.0, 7.9 Hz, 1H), 1.31 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.1, 169.5, 156.0, 149.8, 149.3, 128.0, 107.3, 104.8, 61.7, 56.3, 56.1, 53.6, 30.1, 14.2.

4.1.2. 2-(3-Chloropropyl)-5,6-dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester (15a, *n* = 3)

A mixture of 5,6-dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester **14** (235 mg, 0.88 mmol) and NaH (79.5 mg, 2.65 mmol, 80% dispersion in mineral oil) in DMF (8 mL) was stirred at room temperature for 30 min, after which 1-chloro-3-iodo propane (0.185 mL, 1.76 mmol) was added. The reaction mixture was stirred at room temperature for 20 h and quenched by adding water (15 mL) and aqueous HCl (1 mL, 1.0 N). The mixture was extracted with dichloromethane (3 × 20 mL), and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting residue was

purified by column chromatography (silica gel, EtOAc/PE, 1/4) to generate the title compound (200 mg, 66%) as pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.09 (s, 1H), 6.85 (s, 1H), 4.07 (q, J = 7.1 Hz, 2H), 3.91 (s, 3H), 3.83 (s, 3H), 3.55 (d, J = 17.0 Hz, 1H), 3.44 (t, J = 6.5 Hz, 2H), 2.91 (d, J = 17.0 Hz, 1H), 2.14 (m, 1H), 1.95 (m, 1H), 1.67 (m, 2H), 1.16 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.5, 171.0, 156.0, 149.7, 148.3, 127.6, 107.0, 104.7, 61.4, 60.1, 56.2, 55.9, 44.7, 36.5, 32.0, 27.8, 13.9.

4.1.3. 2-(4-Chlorobutyl)-5,6-dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester (15b, *n* = 4)

Yield 65%. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (s, 1H), 6.84 (s, 1H), 4.07 (q, J = 7.0 Hz, 2H), 3.90 (s, 3H), 3.82 (s, 3H), 3.54 (d, J = 17.0 Hz, 1H), 3.43 (t, J = 6.5 Hz, 2H), 2.91 (d, J = 17.0 Hz, 1H), 1.95–2.05 (m, 1H), 1.65–1.82 (m, 3H), 1.16–1.35 (m, 2H), 1.13 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.7, 171.0, 155.8, 149.5, 148.4, 127.6, 107.0, 104.6, 61.3, 60.6, 56.1, 55.8, 44.3, 36.1, 33.6, 32.4, 21.7, 13.9.

4.1.4. 2-(5-Bromopentyl)-5,6-dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester (15c, *n* = 5)

Yield 50%. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.11 (s, 1H), 6.86 (s, 1H), 4.10 (q, J = 7.0 Hz, 2H), 3.93 (s, 3H), 3.85 (s, 3H), 3.56 (d, J = 17.0 Hz, 1H), 3.31 (t, J = 7.0 Hz, 2H), 2.92 (d, J = 17.0 Hz, 1H), 1.99–2.09 (m, 1H), 1.72–1.88 (m, 3H), 1.34–1.44 (m, 2H), 1.14–1.27 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.9, 171.2, 155.9, 149.5, 148.4, 127.8, 107.0, 104.7, 61.4, 60.7, 56.2, 56.0, 36.3, 34.4, 33.5, 32.2, 28.2, 23.6, 13.9.

4.1.5. 2-(6-Bromohexyl)-5,6-dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester (15d, *n* = 6)

Yield 61%. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.11 (s, 1H), 6.85 (s, 1H), 4.11 (q, J = 7.0 Hz, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.55 (d, J = 17.0 Hz, 1H), 3.31 (t, J = 6.7 Hz, 2H), 2.92 (d, J = 17.0 Hz, 1H), 1.98–2.05 (m, 1H), 1.70–1.86 (m, 3H), 1.14–1.36 (m, 6H), 1.16 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 201.1, 171.2, 155.9, 149.5, 148.5, 127.8, 107.0, 104.7, 61.3, 60.8, 56.2, 56.0, 36.3, 34.5, 33.7, 32.5, 28.9, 27.7, 24.3, 13.9.

4.1.6. 2-(7-Bromoheptyl)-5,6-dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester (15e, *n* = 7)

Yield 54%. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.13 (s, 1H), 6.87 (s, 1H), 4.13 (q, J = 7.0 Hz, 2H), 3.95 (s, 3H), 3.87 (s, 3H), 3.57 (d, J = 17.0 Hz, 1H), 3.34 (t, J = 6.7 Hz, 2H), 2.94 (d, J = 17.0 Hz, 1H), 2.02–2.06 (m, 1H), 1.73–1.84 (m, 3H), 1.14–1.37 (m, 8H), 1.18 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.2, 171.4, 155.9, 149.6, 148.6, 127.9, 107.1, 104.8, 61.4, 60.9, 56.3, 56.1, 36.4, 34.7, 33.9, 32.7, 29.6, 28.4, 27.9, 24.4, 14.0.

4.1.7. 2-(9-Bromononyl)-5,6-dimethoxy-1-oxo-indan-2-carboxylic acid ethyl ester (15g, *n* = 9)

Yield 46%. Pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.16 (s, 1H), 6.89 (s, 1H), 4.14 (q, J = 7.0 Hz, 2H), 3.97 (s, 3H), 3.89 (s, 3H), 3.60 (d, J = 17.0 Hz, 1H), 3.38 (t, J = 6.7 Hz, 2H), 2.96 (d, J = 17.0 Hz, 1H), 2.03–2.07 (m, 1H), 1.77–1.86 (m, 3H), 1.17–1.40 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 201.3, 171.4, 155.9, 149.6, 148.6, 128.0, 107.1, 104.9, 61.5, 61.0, 56.3, 56.1, 36.4, 34.8, 34.0, 32.7, 29.8, 29.3, 29.2, 28.6, 28.1, 24.6, 14.1.

4.1.8. (2*RS*)-2-{3-[(5*S*)-2-Benzyloxy-5,6,7,8-tetrahydro-quinolin-5-ylamino]-propyl}-5,6-dimethoxy-indan-1-one [(*RS,S*)-16a, *n* = 3]

A mixture of **15a**, (200 mg, 0.58 mmol) and **(*S*)-3** (223.8 mg, 0.88 mmol) in toluene (0.5 mL) was refluxed for 20 h. The solvent was removed in vacuo to generate a residue. A mixture of the residue and KOH (240 mg, 4.28 mmol) in ethanol/water (3 mL / 0.5 mL) was refluxed for 21 h. The solvent was removed under reduced pressure and ice water (20 mL) was added. After extraction with dichloromethane (3 × 20 mL), the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, MeOH/DCM, 3/97) to generate the title compound (90 mg, 32% for two steps). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54–7.58 (m, 1H), 7.42–7.44 (m, 2H), 7.28–7.36 (m, 3H), 7.15 (s, 1H), 6.84 (s, 1H), 6.57 (d, J = 8.5 Hz, 1H), 5.31 (s, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.69 (br s, 1H), 3.20 (dd, J = 16.7, 7.0 Hz, 1H), 2.63–2.81 (m, 6H), 1.56–1.98 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.4, 161.7, 155.4, 154.6, 149.3, 148.9, 139.7, 137.5, 129.3, 128.2, 128.2, 128.0, 128.0, 127.6, 127.0, 108.2, 107.3, 104.2, 67.3, 56.1, 56.0, 54.5, 47.3, 46.8, 32.4, 32.2, 29.3, 28.1, 28.0, 18.7.

4.1.9. (2*RS*)-2-{4-[(5*S*)-2-Benzyloxy-5,6,7,8-tetrahydro-quinolin-5-ylamino]-butyl}-5,6-dimethoxy-indan-1-one [(*RS,S*)-16b, *n* = 4]

Yield 45% for two steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, J = 8.5 Hz, 1H), 7.44–7.46 (m, 2H), 7.27–7.37 (m, 3H), 7.17 (s, 1H), 6.86 (s, 1H), 6.59 (d, J = 8.5 Hz, 1H), 5.33 (s, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 3.70 (br s, 1H), 3.22 (dd, J = 16.7, 7.7 Hz, 1H), 2.64–2.84 (m, 6H), 1.48–2.03 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.5, 161.7, 155.4, 154.6, 149.3, 148.9, 139.6, 137.5, 129.4, 128.2, 128.2, 128.0, 128.0, 127.6, 127.1, 108.2, 107.3, 104.2, 67.3, 56.1, 56.0, 54.5, 47.5, 46.7, 32.5, 32.2, 31.5, 30.4, 28.0, 25.1, 18.7.

4.1.10. (2*RS*)-2-{5-[(5*S*)-2-Benzyloxy-5,6,7,8-tetrahydro-quinolin-5-ylamino]-pentyl}-5,6-dimethoxy-indan-1-one [(*RS,S*)-16c, *n* = 5]

Yield 57% for two steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, J = 8.5 Hz, 1H), 7.42–7.45 (m, 2H), 7.27–7.36 (m, 3H), 7.15 (s, 1H), 6.84 (s, 1H), 6.57 (d, J = 8.5 Hz, 1H), 5.32 (s, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.70 (br s, 1H), 3.22 (dd, J = 16.8, 7.2 Hz, 1H), 2.62–2.86 (m, 6H), 1.78–2.01 (m, 6H), 1.41–1.54 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.6, 161.7, 155.3, 154.6, 149.3, 148.9, 139.6, 137.5, 129.4, 128.3, 128.3, 128.0, 128.0, 127.6, 126.9, 108.3, 107.3, 104.2, 67.3, 56.1, 56.0, 54.4, 47.5, 46.7, 32.5, 32.2, 31.5, 30.1, 28.0, 27.3, 27.2, 18.7.

4.1.11. (2*RS*)-2-{6-[(5*S*)-2-Benzyloxy-5,6,7,8-tetrahydro-quinolin-5-ylamino]-hexyl}-5,6-dimethoxy-indan-1-one [(*RS,S*)-16d, *n* = 6)]

Yield 47% for two steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (d, J = 8.5 Hz, 1H), 7.44–7.47 (m, 2H), 7.27–7.39 (m, 3H), 7.17 (s, 1H), 6.87 (s, 1H), 6.59 (d, J = 8.5 Hz, 1H), 5.33 (s, 2H), 3.96 (s, 3H), 3.91 (s, 3H), 3.73 (br s, 1H), 3.22 (dd, J = 16.8, 7.2 Hz, 1H), 2.62–2.88 (m, 6H), 1.74–2.02 (m, 6H), 1.36–1.51 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.7, 161.7, 155.3, 154.6, 149.3, 148.9, 139.6, 137.5, 129.4, 128.3, 128.3, 128.0, 128.0, 127.6, 126.9, 108.3, 107.3, 104.2, 67.3, 56.1, 56.0, 54.4, 47.6, 46.8, 32.5, 32.2, 31.5, 30.2, 29.5, 28.0, 27.3, 27.2, 18.7.

4.1.12. (2*RS*)-2-{7-[(5*S*)-2-Benzyloxy-5,6,7,8-tetrahydro-quinolin-5-yl-amino]-heptyl}-5,6-dimethoxy-indan-1-one [(*RS,S*)-16e, *n* = 7]

Yield 59% for two steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, J = 8.5 Hz, 1H), 7.42–7.44 (m, 2H), 7.26–7.36 (m, 3H), 7.15 (s, 1H), 6.84 (s, 1H), 6.57 (d, J = 8.5 Hz, 1H), 5.31 (s, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.70 (br s, 1H), 3.20 (dd, J = 16.8, 7.2 Hz, 1H), 2.57–2.82 (m, 6H), 1.75–1.98 (m, 6H), 1.31–1.55 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.7, 161.7, 155.3, 154.6, 149.3, 148.9, 139.6, 137.5, 129.4, 128.2, 128.2, 128.0, 128.0, 127.6, 126.9, 108.3, 107.3, 104.2, 67.3, 56.1, 56.0, 54.4, 47.6, 46.8, 32.5, 32.2, 31.5, 30.2, 29.5, 29.3, 28.0, 27.3, 27.2, 18.7.

4.1.13. (2*RS*)-2-{9-[(5*S*)-2-Benzyloxy-5,6,7,8-tetrahydroquinolin-5-ylamino]-nonyl}-5,6-dimethoxy-indan-1-one [(*RS,S*)-16g, *n* = 9]

Yield 42% for two steps. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.55 (d, J = 8.5 Hz, 1H), 7.42–7.44 (m, 2H), 7.26–7.36 (m, 3H), 7.15 (s, 1H), 6.84 (s, 1H), 6.57 (d, J = 8.5 Hz, 1H), 5.31 (s, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.70 (br s, 1H), 3.20 (dd, J = 16.8, 7.2 Hz, 1H), 2.58–2.80 (m, 6H), 1.76–2.02 (m, 6H), 1.19–1.52 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.8, 161.7, 155.4, 154.7, 149.3, 148.9, 139.7, 137.5, 129.4, 128.3, 128.3, 128.0, 128.0, 127.6, 126.9, 108.3, 107.3, 104.2, 67.3, 56.1, 56.0, 54.4, 47.6, 46.8, 32.5, 32.2, 31.6, 30.2, 29.6, 29.5, 29.4, 29.4, 27.9, 27.3, 27.3, 18.7.

4.1.14. (5*S*)-5-{3[(2*RS*)-5,6-Dimethoxy-1-oxo-indan-2-yl]-propylamino}-5,6,7,8-tetrahydro-1*H*-quinolin-2-one [(*RS,S*)-17a]

To a solution of **(*RS,S*)-16a** (68 mg, 0.14 mmol) in MeOH (2 mL), 10% Pd/C (40 wt%, 27 mg) was added, and the mixture was stirred under hydrogen atmosphere at room temperature for 3 h. The catalyst was removed by filtration on Celite and the filtrate was concentrated under vacuum. The resulting residue was purified by column chromatography (silica gel, MeOH/DCM/$NH_3{\cdot}H_2O$, 1/19/0.2) to generate the title compound (50 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43–7.48 (m, 1H), 7.12 (s, 1H), 6.82 (s, 1H), 6.35 (d, J = 8.7 Hz, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.51 (br s, 1H), 3.20 (dd, J = 16.8, 7.2 Hz, 1H), 2.58–2.73 (m, 6H), 1.54–1.94 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.4, 165.0, 155.4, 149.4, 148.9, 144.3, 143.3, 129.3, 117.3, 116.9, 107.3, 104.2, 56.1, 56.0, 53.0, 47.4, 46.9, 32.4, 29.3, 28.1, 27.5, 26.7, 18.7. MS (HRCI) calcd for $C_{23}H_{29}N_2O_4$ $[M+H]^+$ 397.2122; Found 397.2136. $[\alpha]_D^{25}$ −27.7° (c 0.57, MeOH)

4.1.15. (5*S*)-5-{4-[(2*RS*)-5,6-Dimethoxy-1-oxo-indan-2-yl]-butylamino}-5,6,7,8-tetrahydro-1*H*-quinolin-2-one [(*RS,S*)-17b]

Yield 91%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (d, J = 9.0 Hz, 1H), 7.13 (s, 1H), 6.83 (s, 1H), 6.36 (d, J = 9.0 Hz, 1H), 3.93 (s, 3H), 3.87 (s, 3H), 3.51 (br s, 1H), 3.20 (dd, J = 16.8, 7.2 Hz, 1H), 2.56–2.72 (m, 6H), 1.68–1.90 (m, 6H), 1.45–1.54 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.6, 165.0, 155.4, 149.4, 148.9, 144.3, 143.3, 129.4, 117.4, 116.9, 107.3, 104.2, 56.1, 56.0, 52.9, 47.5, 46.7, 32.5, 31.5, 30.4, 27.5, 26.7, 25.0, 17.3. MS (HRCI) calcd for $C_{24}H_{31}N_2O_4$ $[M+H]^+$ 411.2278; Found 411.2284. $[\alpha]_D^{23}$ −39.3° (c 2.62, MeOH).

4.1.16. (5*S*)-5-{5-[(2*RS*)-5,6-Dimethoxy-1-oxo-indan-2-yl]-pentylamino}-5,6,7,8-tetrahydro-1*H*-quinolin-2-one [(*RS,S*)-17c]

Yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43 (d, J = 9.3 Hz, 1H), 7.11 (s, 1H), 6.82 (s, 1H), 6.33 (d, J = 9.3 Hz, 1H), 3.90 (s, 3H), 3.84 (s, 3H), 3.49 (br s, 1H), 3.17 (dd, J = 16.8, 7.2 Hz, 1H), 2.51–2.69 (m, 6H), 1.70–1.87 (m, 6H), 1.42–1.49 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.6, 165.0, 155.3, 149.2, 148.9, 144.2, 143.2, 129.3, 117.3, 116.8, 107.3, 104.2, 56.1, 56.0, 52.8, 47.5, 46.8, 32.5, 31.5, 30.2, 27.3, 27.2, 27.1, 26.6, 17.3. MS (HRCI) calcd for $C_{25}H_{33}N_2O_4$ $[M+H]^+$ 425.2435; Found 425.2446. $[\alpha]_D^{23}$ −38.6° (c 0.66, MeOH).

4.1.17. (5*S*)-5-{6-[(2*RS*)-5,6-Dimethoxy-1-oxo-indan-2-yl]-hexylamino}-5,6,7,8-tetrahydro-1*H*-quinolin-2-one [(*RS,S*)-17d]

Yield 94%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 (d, J = 9.3 Hz, 1H), 7.18 (s, 1H), 6.88 (s, 1H), 6.40 (d, J = 9.3 Hz, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.54 (br s, 1H), 3.22 (dd, J = 16.8, 7.2 Hz, 1H), 2.58–2.76 (m, 6H), 1.77–1.92 (m, 6H), 1.41–1.49 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.7, 165.1, 155.3, 149.2, 148.9, 144.2, 143.3, 129.4, 117.4, 116.8, 107.3, 104.2, 56.1, 56.0, 52.8, 47.5, 47.0, 32.5, 31.5, 30.3, 29.4, 27.4, 27.2, 27.2, 26.7, 17.3. MS (HRCI) calcd for $C_{26}H_{35}N_2O_4$ $[M+H]^+$ 439.2591; Found 439.2608. $[\alpha]_D^{23}$ −14.0° (c 0.29, MeOH).

4.1.18. (5*S*)-5-{7-[(2*RS*)-5,6-Dimethoxy-1-oxo-indan-2-yl]-heptylamino}-5,6,7,8-tetrahydro-1*H*-quinolin-2-one [(*RS,S*)-17e]

Yield 90%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (d, J = 9.0 Hz, 1H), 7.09 (s, 1H), 6.80 (s, 1H), 6.32 (d, J = 9.0 Hz, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 3.48 (br s, 1H), 3.15 (dd, J = 16.8, 7.2 Hz, 1H), 2.49–2.68 (m, 6H), 1.69–1.86 (m, 6H), 1.26–1.47 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.6, 165.0, 155.2, 149.2, 148.9, 144.2, 143.2, 129.3, 117.3, 116.7, 107.2, 104.1, 56.0, 55.9, 52.8, 47.5, 46.9, 32.4, 31.5, 30.3, 29.4, 29.2, 27.3, 27.2, 27.2, 26.6, 17.2. MS (HRCI) calcd for $C_{27}H_{37}N_2O_4$ $[M+H]^+$ 453.2748; Found 453.2743. $[\alpha]_D^{23}$ −29.5° (c 1.00, MeOH).

4.1.19. (5*S*)-5-{9-[(2*RS*)-5,6-Dimethoxy-1-oxo-indan-2-yl]-nonylamino}-5,6,7,8-tetrahydro-1*H*-quinolin-2-one [(*RS,S*)-17g]

Yield 94%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47 (d, J = 9.0 Hz, 1H), 7.15 (s, 1H), 6.85 (s, 1H), 6.37 (d, J = 9.0 Hz, 1H), 3.94 (s, 3H), 3.88 (s, 3H), 3.54 (br s, 1H), 3.20 (dd, J = 16.8, 7.2 Hz, 1H), 2.55–2.73 (m, 6H), 1.70–1.91 (m, 6H), 1.26–1.47 (m, 14H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 207.8, 165.0, 155.4, 149.3, 149.0, 144.3, 143.3, 129.5, 117.5, 116.9, 107.4, 104.3, 56.2, 56.1, 53.0, 47.7, 47.0, 32.6, 31.7, 30.3, 29.6, 29.5, 29.5, 29.4, 27.3, 27.3, 27.3, 26.8, 17.4. MS (HRCI) calcd for $C_{29}H_{41}N_2O_4$ $[M+H]^+$ 481.3061; Found 481.3061. $[\alpha]_D^{23}$ −24.3° (c 0.82, MeOH).

4.1.20. (5*R*)-5-{4-[(2*RS*)-5,6-Dimethoxy-1-oxo-indan-2-yl]-butylamino}-5,6,7,8-tetrahydro-1*H*-quinolin-2-one [(*RS,R*)-17b]

Procedures were the same as **(*RS,S*)-17b** except that **(*R*)-3** was the starting material instead of **(*S*)-3**. HRMS (ESI) calcd for $C_{24}H_{31}N_2O_4$ $[M+H]^+$ 411.2284; Found 411.2291. $[\alpha]_D^{23}$ +36.6° (c 1.16, MeOH).

4.2. In vitro cholinesterase inhibition studies

Sprague–Dawley rats of 3–6 week-old were supplied by the Animal Care Facility and the procedures used were approved by the Ethics Committee of The Hong Kong University of Science and Technology. All the reagents were purchased from Sigma. Brain cortices and serum collected from rats immediately after decapitation were used as sources of AChE and BuChE, respectively. The traditional colorimetric method (Ellman's method)[23] was used for the detection of cholinesterase activity and modified for use in 96-well-plates (NUNC) with a final volume of 200 μL. Briefly, the cholinesterase inhibitory activity of the potential compounds inhibits the breakdown of the substrate (acetyl thiocholine iodide) to acetic acid and choline, a reaction catalyzed by the enzymatic action of AChE. Acetic acid and the chromogen 5, 5′-dithio-bis(2-nitrobenzoic) acid react to form yellow anions of 5-thio-2-nitro-benzoic acid that are then detected in a plate reader at an absorbance of 405 nm (ref λ 630 nm). Ethopropazine–HCl (100 μM) and BW284c51 (10 μM) were added to the brain homogenate and serum, respectively. The inhibitory curve and the IC_{50} were determined from series of drug concentrations using GraphPad Prism 5. Assays were performed in duplicate and the values were presented as the mean ± SEM from three independent experiments. The specificity of the inhibition was calculated as a ratio by comparing the inhibition potency of BuChE over AChE.

Acknowledgements

We would like to thank Dr. Guangmiao Fu for his critical reading of the manuscript and members of the Ip laboratory for their helpful comments and suggestions. This study was supported by the Area of Excellence Scheme of the University Grants Committee of HKSAR (AoE/B-15/01), Hong Kong Jockey Club Charities Trust

and the Innovation and Technology Fund of Hong Kong (ITS/246/09FP) with sponsorship by Axis Biotech Pty Limited.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.11.044.

References and notes

1. Cummings, J. L.; Askin-Edgar, S. *CNS Drugs* **2000**, *13*, 385.
2. Batsch, N. L.; Mittelman, M. S. *World Alzheimer Report* **2012**, 5.
3. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. *Science* **1982**, *217*, 408.
4. Thies, W.; Bleiler, L. *Alzheimers Dement.* **2012**, *8*, 131.
5. Munoz-Torrero, D.; Camps, P. *Curr. Med. Chem.* **2006**, *13*, 399.
6. Pang, Y.-P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S. *J. Biol. Chem.* **1996**, *271*, 23646.
7. Carlier, P. R.; Du, D. M.; Han, Y. F.; Liu, J.; Perola, E.; Williams, I. D.; Pang, Y. P. *Angew. Chem., Int. Ed.* **2000**, *39*, 1775.
8. Carlier, P. R.; Du, D.-M.; Han, Y.; Liu, J.; Pang, Y.-P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2335.
9. Alonso, D.; Dorronsoro, I.; Rubio, L.; Muñoz, P.; García-Palomero, E.; Del Monte, M.; Bidon-Chanal, A.; Orozco, M.; Luque, F. J.; Castro, A.; Medina, M.; Martínez, A. *Bioorg. Med. Chem.* **2005**, *13*, 6588.
10. Shao, D.; Zou, C.; Luo, C.; Tang, X.; Li, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4639.
11. Camps, P.; Formosa, X.; Galdeano, C.; Gomez, T.; Munoz-Torrero, D.; Scarpellini, M.; Viayna, E.; Badia, A.; Clos, M. V.; Camins, A.; Pallas, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Estelrich, J.; Lizondo, M.; Bidon-Chanal, A.; Luque, F. J. *J. Med. Chem.* **2008**, *51*, 3588.
12. Bolea, I.; Juarez-Jimenez, J.; Rios, C.; Chioua, M.; Pouplana, R.; Luque, F. J.; Unzeta, M.; Marco-Contelles, J.; Samadi, A. *J. Med. Chem.* **2011**, *54*, 825.
13. Tumiatti, V.; Minarini, A.; Bolognesi, M. L.; Milelli, A.; Rosini, M.; Melchiorre, C. *Curr. Med. Chem.* **1825**, *2010*, 17.
14. Viayna, E.; Gomez, T.; Galdeano, C.; Ramirez, L.; Ratia, M.; Badia, A.; Clos, M. V.; Verdaguer, E.; Junyent, F.; Camins, A.; Pallas, M.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M. P.; Rodríguez-Franco, M. I.; Bidon-Chanal, A.; Luque, F. J.; Camps, P.; Munoz-Torrero, D. *ChemMedChem* **1855**, *2010*, 5.
15. Monte-Millan, M.; Garcia-Palomero, E.; Valenzuela, R.; Usan, P.; de Austria, C.; Munoz-Ruiz, P.; Rubio, L.; Dorronsoro, I.; Martinez, A.; Medina, M. *J. Mol. Neurosci.* **2006**, *30*, 85.
16. Koellner, G.; Kryger, G.; Millard, C. B.; Silman, I.; Sussman, J. L.; Steiner, T. *J. Mol. Biol.* **2000**, *296*, 713.
17. Saxena, A.; Fedorko, J. M.; Vinayaka, C. R.; Medhekar, R.; Radić, Z.; Taylor, P.; Lockridge, O.; Doctor, B. P. *Eur. J. Biochem.* **2003**, *270*, 4447.
18. Kryger, G.; Silman, I.; Sussman, J. L. *Structure* **1999**, *7*, 297.
19. Raves, M. L.; Harel, M.; Pang, Y. P.; Silman, I.; Kozikowski, A. P.; Sussman, J. L. *Nat. Struct. Biol.* **1997**, *4*, 57.
20. Wong, D. M.; Greenblatt, H. M.; Dvir, H.; Carlier, P. R.; Han, Y.-F.; Pang, Y.-P.; Silman, I.; Sussman, J. L. *J. Am. Chem. Soc.* **2003**, *125*, 363.
21. Kawakami, Y.; Inoue, A.; Kawai, T.; Wakita, M.; Sugimoto, H.; Hopfinger, A. J. *Bioorg. Med. Chem.* **1996**, *4*, 1429.
22. (a) Greenhill, J. V. *J. Chem. Soc. C* **1971**, 2699; b) Pettit, G. R.; Fleming, W. C.; Paull, K. D. *J. Org. Chem.* **1968**, *33*, 1089.
23. Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
24. Sugimoto, H.; Yamanishi, Y.; Iimura, Y.; Kawakami, Y. *Curr. Med. Chem.* **2000**, *7*, 303.
25. Cardozo, M. G.; Iimura, Y.; Sugimoto, H.; Yamanishi, Y.; Hopfinger, A. J. *J. Med. Chem.* **1992**, *35*, 584.