Journal of Medicinal Chemistry

Article

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Synthesis of Monomeric Derivatives To Probe Memoquin's Bivalent Interactions

Maria Laura Bolognesi,*[,†] GianPaolo Chiriano,[‡] Manuela Bartolini,[†] Francesca Mancini,[†] Giovanni Bottegoni,[§] Valentina Maestri,[†] Stefan Czvitkovich,[∥] Manfred Windisch,[∥] Andrea Cavalli,[†,§] Anna Minarini,[†] Michela Rosini,[†] Vincenzo Tumiatti,[†] Vincenza Andrisano,[†] and Carlo Melchiorre[†]

[†]Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

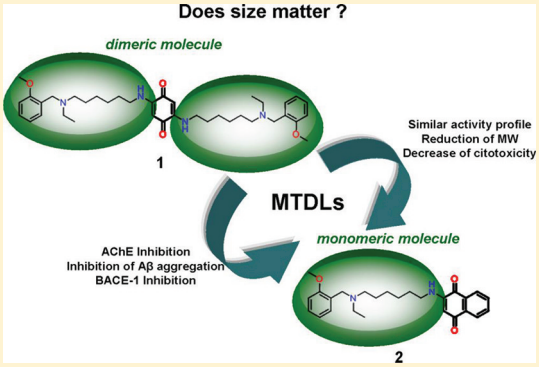
[‡]Statistical and Biological Physics Sector, SISSA-ISAS, Via Bonomea 265, 34136 Trieste, Italy

[§]Department of Drug Discovery and Development, Italian Institute of Technology, Via Morego 30, 16163 Genova, Italy

[∥]JSW Lifesciences GmbH, Parkring 12, 8074 Grambach, Austria

Supporting Information

ABSTRACT: Eight monomeric congeners, related to the multitarget lead candidate memoquin, were prepared and evaluated at multiple targets to determine their profile against Alzheimer's disease. **2**–**4** bind to AChE with similar low nanomolar affinities and function as effective inhibitors of amyloid aggregation. The most potent monovalent ligand **2** also inhibits BACE-1 in vitro and APP metabolism in primary chicken telencephalic neurons.



INTRODUCTION

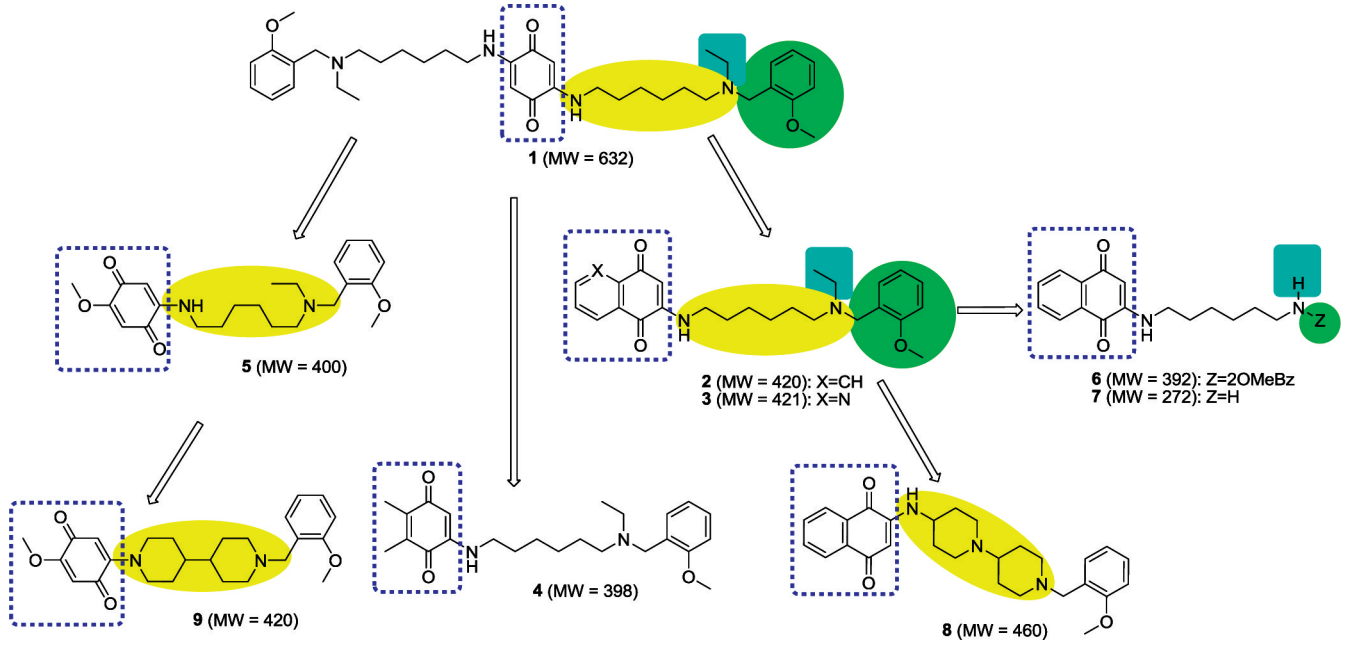
Memoquin (**1**) was rationally designed to create a new chemical entity (NCE) with a polypharmacological profile against Alzheimer's disease (AD).[1,2] An in vitro and in vivo characterization revealed its multifunctional mechanism of action and its interaction with three molecular targets involved in AD pathology, namely, acetylcholinesterase (AChE), β-amyloid (Aβ), and β-secretase (BACE-1).[3] **1** is thus the successful product of one of the first AD multitarget drug discovery efforts. This strategy is emerging as an alternative way to develop effective anti-AD drugs.[4−7] Although the single-target approach remains the main strategy in big pharmaceutical companies, the ongoing failure of current candidates is convincing the pharmaceutical community that the desired outcome may be provided more effectively by a drug that modulates multiple targets.[8] From a medicinal chemistry point of view, **1** is a bivalent ligand, with a symmetrical structure composed of two 2-methoxybenzyldiamino moieties connected by a benzoquinone spacer. Several examples of linking two pharmacophoric units via spacers of different length and flexibility were reported by Portoghese in the field of opioids.[9] In AD too, the bivalent ligand strategy has received attention over the past decade.[10−12] This was mostly motivated by the peculiar topology of a classical AD target, the enzyme AChE, which has two recognition sites sharing common molecular features.[13] Consequently, improved potency is shown by drugs that simultaneously bind the catalytic and the peripheral anionic (PAS) sites of AChE.[14−18]

The multitarget approach could be considered an evolution of the bivalent ligands concept. This is because combining structural elements from two ligands is the simplest way of incorporating activity at two targets into a single molecule.[19] The rationale for using the bivalent ligand approach in AD also stems from the possibility that dimeric structures may be capable of bridging independent recognition sites on other validated targets (such as Aβ and BACE-1), resulting in a binding interaction that is thermodynamically more favorable than the monovalent binding of two molecules. In principle, this would be particularly advantageous in view of the complexity of the recognition mechanism of protein−protein interactions in amyloidosis.[20] As a matter of fact, several amyloid binding compounds share a common bivalent structure and bivalent "molecular tweezers" have been envisaged as the next generation of ligands.[21] Another positive feature of anti-AD bivalent ligands is that because of their high hindrance, they can efficiently fit the extended substrate binding site of BACE-1.[22] However, their high molecular weight (MW) has negative consequences for the pharmacokinetic profile.

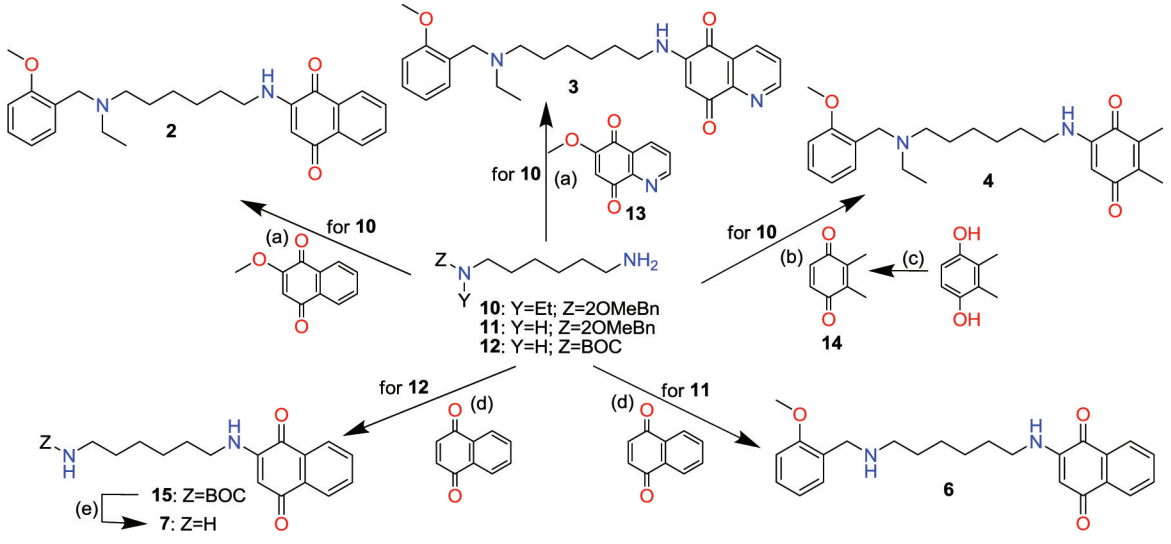
For **1**, we could verify absorption through oral administration and access to the central nervous system.[23] Nevertheless, if we

Received: May 31, 2011
Published: November 7, 2011

Chart 1. Design Strategy for Monovalent Ligands 2−9



Scheme 1[a]



[a]Reagents and conditions: (a) EtOH, 4 h, reflux; (b) CH_2Cl_2, 3 h, air, room temp; (c) MnO_2, dry Et_2O, 4 h, room temp; (d) MeOH, 4 h, air, room temp; (e) TFA, $CHCl_3$, 5 h, room temp.

strictly reason in terms of Lipinski's rule,[24] **1** violates the MW parameter, being out of range (632 vs 500). On this basis, we sought to generate analogues of **1** with a reduced MW, yet maintaining its promising multitarget profile.

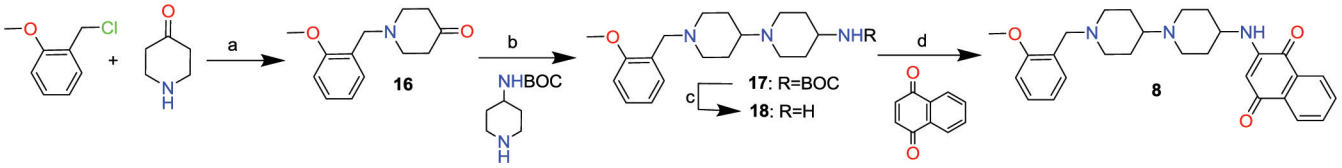
RESULTS AND DISCUSSION

To this end, we prepared and then evaluated at multiple targets (AChE, Aβ, BACE-1) eight congeners (**2**−**9**), which we formally obtained by cutting the dimeric structure of **1** in two halves. In the resulting monomeric compounds **2**−**5**, one of the two 2-methoxybenzyldiamino chains of **1** is always preserved, with the ending fragments being a quinone, as a privileged motif for modulating protein−protein interactions.[25−27] The selected quinones were a napthoquinone (**2**), a quinolinoquinone (**3**), a 2,3-dimethylbenzoquinone (**4**), and a 2-methoxybenzoquinone (**5**). Conversely, in **6** and **7** a structural simplification has been performed to investigate the role of the ethyl- and 2-methoxybenzyl substituents on the basic nitrogen of **2**. Moreover, we reduced the flexibility at the polymethylene chain of **2** and **5** because conformationally restricted analogues **8** and **9** might show improved potency. Notably, all compounds displayed a MW below the 500 Da cutoff (see Chart 1 for design strategy).

In previous studies, we verified that 1,4-Michael addition or substitution reactions would allow us to effectively incorporate a diamine chain into the 2-position of a quinone.[1,2,28] In the cases of **2** and **3**, we used a substitution reaction, which also led to the unambiguous synthesis of **3**. Thus, treating 2-methoxynaphtalene-1,4-dione or 6-methoxyquinoline-5,8-dione (**13**)[29] with diamine **10**[1,2] gave target compounds in

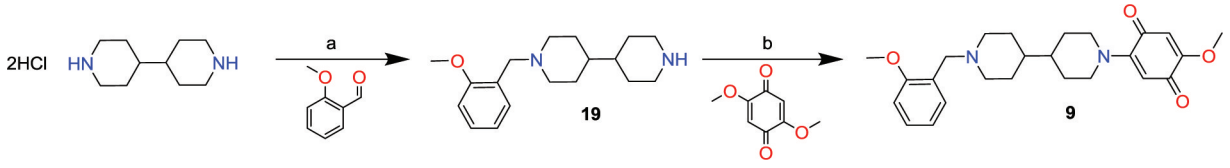
dx.doi.org/10.1021/jm200691d | J. Med. Chem. 2011, 54, 8299−8304

Scheme 2[a]



[a]Reagents and conditions: (a) K_2CO_3, KI, DMF, 4 h, reflux; (b) dry THF, $(CH_3COO)_3BHNa$, CH_3COOH, N_2, overnight, room temp; (c) TFA, dry CH_2Cl_2, 5 h, room temp; (d) MeOH, 5 h, air, room temp.

Scheme 3[a]



[a]Reagents and conditions: (a) $NaBH_3CN$, KOH, MeOH, 3 h, room temp; (b) $CHCl_3$, 72 h, reflux.

Table 1. Inhibitory Activity on Human AChE and BuChE, BACE-1, and Amyloid Aggregation by 2−9 and Reference Compound 1

	IC_{50} (nM)[a]			inhibition of Aβ aggregation (%)	
compd	hAChE	hBuChE	inhibition of BACE-1 (%)[b]	AChE-induced[c]	self-induced[d]
1	1.55 ± 0.11	144 ± 100	>80	87.1 ± 1.7	66.8 ± 4.4
2	9.73 ± 0.44	1490 ± 100	60.2 ± 1.6	69.1 ± 3.2	27.3 ± 4.3
3	27.9 ± 1.6	2560 ± 170	na	41.3 ± 0.7	15.4 ± 6.7
4	29.0 ± 4.0	314 ± 21	12.8 ± 2.0	60.6 ± 0.2	30.2 ± 1.4
5	65.3 ± 2.2	22800 ± 1600	32.8 ± 2.4	nt	45.7 ± 3.4
6	1850 ± 30	3320 ± 260	na	nt	29.5 ± 0.9
7	64500 ± 2700	294000 ± 25000	na	nt	13.8 ± 4.1
8	17200 ± 1000	3160 ± 130	na	nt	21.4 ± 1.3
9	24400 ± 1400	3580 ± 150	32.9 ± 1.0	nt	20.5 ± 1.0

[a]Human recombinant AChE and BuChE from human serum were used. IC_{50} ± SEM values represent the concentration of inhibitor required to decrease enzyme activity by 50% and are the mean of two independent measurements, each performed in duplicate. [b]Inhibition of BACE-1. The concentration of the tested inhibitor was 3 μM. Experimental conditions are as in ref 22. For **2** IC_{50} = 2.8 ± 0.1 μM. IC_{50} represents the concentration of inhibitor required to decrease enzyme activity by 50% and is the mean of three independent measurements, each performed in duplicate; na = not active. [c]Inhibition of AChE-induced $A\beta_{40}$ aggregation. The concentration of the tested inhibitor and $A\beta_{40}$ was 100 and 230 μM, respectively, whereas the $A\beta_{40}$/AChE ratio was equal to 100:1. Values are the mean of two independent experiments each performed in duplicate; nt = not tested. [d]Inhibition of 50 μM $A\beta_{42}$ self-aggregation when [I] = 10 μM was used. The $A\beta_{42}$/inhibitor ratio was equal to 5:1. Values are the mean of two independent experiments each performed in duplicate.

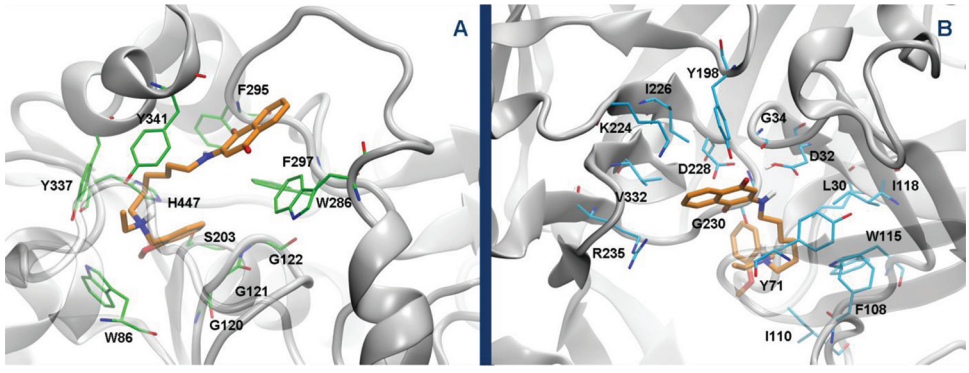


Figure 1. Low energy docking model of **2** (orange) into the active sites of AChE (A, residues displayed in light green) and BACE-1 (B, residues displayed in light cyan).

good yields. Conversely, **4** was synthesized by reacting **10** with quinone **14**, obtained, in turn, by oxidation of 2,3-dimethylbenzene-1,4-diol. Similarly, Michael addition of amines **11**[2] and **12** afforded **6** and **15**, respectively. Subsequent deprotection of BOC protecting group of **15** led to final compound **7** (Scheme 1). Michael addition was also used to

dx.doi.org/10.1021/jm200691d | J. Med. Chem. 2011, 54, 8299–8304

synthesize **8**, starting from naphthoquinone and **18** (Scheme 2). The amine **18** was derived by reductive amination between the commercially available *tert*-butyl piperidin-4-ylcarbamate and **16**, obtained by a nucleophilic substitution reaction, followed by BOC removal. Finally, to obtain **9**, monosubstitution of 2,5-dimethoxy-1,4-benzoquinone with diamine **19**, synthesized through an efficient reductive amination protocol, was carried out as reported for **5**[30] (Scheme 3).

To characterize the multitarget profiles of **2**–**9**, their inhibitory activity at human AChE and butyrylcholinesterase (BuChE) (Table 1) was tested. Kinetic and molecular modeling evidence has demonstrated that **1** is a dual binding cholinesterases inhibitor, which accounts for its remarkable nanomolar activity.[1,2] Interestingly, all compounds, except **6**–**9** were effective inhibitors of AChE, with **2** being just 6 times less potent than **1**. This suggests that even the monovalent structures of **2**–**5** could establish interactions with both sites of the enzyme. However, the low activity displayed by **6** and **7** confirms the importance of an ethyl and a 2-methoxybenzyl group as substituents on the terminal protonable nitrogen, as verified for **1** derivatives.[2,27] It is also plausible that the constrained structures of **8** and **9** did not allow an optimal docking into the AChE gorge. Figure 1A reports the bound conformation of **2**, resulting from docking simulations carried out at the active site of hAChE (PDB code 1B41; see Supporting Information). Here, we see that **2** was able to interact with the catalytic region and at the same time to protrude toward the solvent-exposed gorge entrance. The following interactions between **2** and hAChE were observed: (i) the ligand protonated nitrogen established a cation–π interaction with the indole ring of W86 and the phenol ring of Y337; (ii) the oxygen in position 1 of the quinone moiety established an H-bond interaction with the backbone of F295; (iii) the naphthalenedione moiety established a favorable π–π stacking with the indole ring of W286 of the PAS. This last finding was relevant in the context of previous reports that linked inhibition of AChE-induced Aβ aggregation with a binder's ability to interact with the PAS of the enzyme.[31,32]

Indeed, the AChE-induced Aβ aggregation experiments,[31,32] performed on the three most active AChEIs **2**–**4**, are in agreement with the proposed binding mode at AChE. Activities in the AChE-induced aggregation and AChE inhibition were highly correlated for **2**, which was the most potent in both assays. The IC_{50} for the inhibition of the AChE-induced Aβ aggregation by **2** was also calculated, being 45 ± 6 μM. A different pattern was found for **4**, which is the less potent against AChE, while showing an intermediate antiaggregation activity. Concerning the physiological relevance of these in vitro studies, it must be taken into account that the AChE concentration in the induced Aβ aggregation assay is roughly 1000–3000 times higher than that in the cerebrospinal fluid of AD patients.[33] Consequently, inhibitory activity reported in Table 1 refers to a normalized inhibitor concentration of 35–100 nM, a concentration that is closely correlated with those needed to exert the other activities.

In light of the remarkable antiaggregating properties of **1**[34] and several other bivalent ligands,[22,35] the ability of **2**–**9** to reduce $A\beta_{42}$ spontaneous aggregation was then investigated. Data in Table 1 show that **2**–**9** at 10 μM inhibited Aβ self-aggregation in a range from 14% to 46%. At the same concentration, **1** displayed 68%, which is less than 2 times higher than that of the most potent compound **5**. Interestingly, two protonable diamino chains of **1** do not appear to be necessary for Aβ binding. Conversely, these data pinpoint to the quinone core as an essential feature for potent aggregation inhibition, in agreement with the well-documented inhibitory capability of quinones toward Aβ assembly.[25–27,36–38] Spacer flexibility also seems to be important, with **9** showing halved percent inhibition with respect to **5**. When rigid **8** is compared to flexible **2**, the relatively lower reduction in potency (29% vs 22%, respectively) might be attributed to the concomitant presence in **8** of an additional protonable nitrogen atom, which could establish additional positive interactions.

As part of its multitarget profile, **1** inhibits BACE-1 quite effectively.[1] Therefore, preliminary studies were carried out to assess whether the monomeric derivatives retained the ability to inhibit BACE-1 in vitro. **2**–**9** were tested at 3 μM, and their inhibition percentages are reported in Table 1 in comparison to **1** (80%). The most potent compounds were **2**, **5**, and **9**, which inhibited enzyme activity at 32–60%, whereas **3** and **6**–**8** showed no activity. For **2** we also determined an IC_{50} of 2.8 μM. To check for nonspecific effects, additional experiments were performed on **1** and **2** with a different source of enzyme and type of substrate and in the presence of a detergent (see Supporting Information). In this second assay, **2** showed a similar potency (IC_{50} = 3.0 μM) while **1** was less active (IC_{50} = 4.6 μM). The discrepancy of results between the two assays for **1** is likely to be a consequence of differences in the substrate, protein, and assay buffer. Docking simulations were performed in an attempt to provide a plausible binding mode compatible with the BACE-1 inhibitory profile of **2**. Figure 1B reports the binding mode of **2** at BACE-1 proteasic site (PDB code 2QZL). The leading interactions that characterized the bound complex were the following: (i) the proximal nitrogen of the spacer established two H-bond interactions with the side chain of catalytic D32 and with the carbonyl oxygen of G34 backbone; (ii) the oxygen in position 4 of naphthalenedione moiety interacted via H-bond with the side chain of Y198; (iii) the protonated nitrogen established an H-bond interaction with the carbonyl oxygen of G230 backbone; (iv) the aliphatic chain of the spacer was lodged in a hydrophobic subpocket described by Y71, F108, I110, L30, I118, and W115; (v) the quinone formed hydrophobic contacts with I226 and V332. In summary, in the reported bound conformation, **2** contacted directly the catalytic dyad and several subsites that have been previously exploited to achieve inhibitory potency.[39]

To further substantiate the secretase inhibitory activity of **2**, **5**, and **9**, we also tested whether they affect APP processing in a cellular context. This study was carried out in primary chicken telencephalon neurons to assess the effect on secretion of $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$.[40] Thus, intrinsic cell toxicity of **2**, **5**, and **9** was first evaluated, using **1** as reference compound. Treating primary neurons for 24 h with **2** (0.01–50 μM) did not lead to modified viability, whereas treatment with high concentrations of **5** (50 μM), **1** (25 μM), and **9** (25 μM) significantly abolished neuronal viability (see Supporting Information). Notably, **1** exhibited a similar toxicity in the SH-SY5Y cell line.[27] These data are encouraging because they point to a molecule-based toxicity rather than to a potential mechanism of toxicity related to the quinone moiety. Concerning studies on amyloid peptides production, **2** inhibited $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ secretion, with IC_{50} values of 19, 21, and 46 μM, respectively. These values were corrected with mean neurons viability, obtained in the MTT reduction assay. Conversely, because of their toxicity, a clear concentration-dependent decrease in Aβ

secretion could not be observed for **1**, **5**, and **9** (see Supporting Information).

There is a growing body of evidence that multitarget-directed ligands (MTDLs)[4] provide a viable area for AD drug discovery. One limitation to this approach is that most of the hits discovered so far tend to have high MW, resulting in new NCEs that are eventually associated with poor oral bioavailability.[41] The low ligand efficiency of MTDLs is a critical issue. This is because affinity at the different targets usually parallels the increase in MW, whereas pharmacokinetic properties are improved by reducing the MW.[41] However, small molecule MTDLs (MW ≈ 500) have been reported.[42] Interestingly, in the present series of **1** derivatives, the reduction of MW did not necessarily correspond to a reduction in the multiple activities. In fact, for the most active compound **2**, the MTDL activity profile remains almost unchanged with respect to **1**, with the toxicity profile actually being improved.

In conclusion, if the decrease in MW translates into the expected superior bioavailability, **2** could be a promising starting point in the search for new MTDLs against AD.

EXPERIMENTAL SECTION

Chemistry. All starting reagents were of the best grade available from Aldrich and TCI. Melting points were taken in glass capillary tubes on Buchi SMP-20 apparatus and are uncorrected. Direct infusion ESI-MS spectra were recorded on Waters ZQ 4000 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded either at 200 MHz (^{1}H) and 50.3 MHz (^{13}C) or at 400 MHz (^{1}H) and 100 MHz (^{13}C). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), brs (broad singlet), d (doublet), t (triplet), or m (multiplet). Elemental analysis was used to confirm ≥95% sample purity, and the elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separations were performed on silica gel columns by flash chromatography (Kieselgel 40, 0.040−0.063 mm; Merck). Reactions were followed by thin-layer chromatography (TLC) on Merck (0.25 mm) glass-packed precoated silica gel plates (60 F254), then visualized in an iodine chamber or with a UV lamp. Compounds were named following IUPAC rules as applied by Beilstein-Institute AutoNom (version 2.1), a PC integrated software package for systematic names in organic chemistry.

Synthesis of 2. The 2-methoxynaphthalene-1,4-dione (0.15 g, 0.83 mmol) was dissolved in EtOH (6 mL) at reflux temperature. To this suspension, a solution of **10**[1,2] (0.2 g, 0.76 mmol) in EtOH (6 mL) was added. The resulting reaction mixture was stirred at reflux temperature for 4 h. The solvent was evaporated under vacuo, and the final red crude product was purified by gravity chromatography (CH_2Cl_2/EtOH/aqueous 33% ammonia = 9/1/0.03). Compound **2** (0.11 g) was obtained as a red solid. Yield 34%; mp = 72 °C. ESI-MS (m/z): 421 (M + H$^+$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08 (d, J = 8.4, 1H), 8.02 (d, J = 8.4, 1H), 7.71 (t, J = 7.2, 1H) 7.59 (t, J = 7.2, 1H), 7.41−7.39 (m, 1H), 7.19 (t, J = 7.2, 1H), 6.92 (t, J = 6.8, 1H), 6.83 (d, J = 8.0, 1H), 5.85 (br s exch, 1H), 5.70 (s, 1H), 3.80 (s, 3H), 3.59 (br s, 2H), 3.12 (q, J = 6.4, 2H), 2.53−2.50 (m, 2H), 2.49−2.46 (m, 2H), 1.67−1.62 (m, 2H), 1.54−1.46 (m, 2H), 1.38−1.30 (m, 4H), 1.13−0.95 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.9, 181.9, 157.7, 147.9, 134.7, 133.7, 131.9, 130.5, 130.1, 126.2, 126.1, 120.3, 110.2, 100.7, 100.6, 55.3, 53.2, 51.3, 47.7, 42.5, 28.2, 27.1, 26.9, 15.2, 11.7. Anal. Calcd for $C_{26}H_{32}N_2O_3$ (420.54): C, 74.26; H, 7.67; N, 6.66. Found: C, 74.60; H, 7.29; N, 6.31.

ASSOCIATED CONTENT

Supporting Information

Experimental details of the synthesis of **2**−**9** and computational and biological methods. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +39 0512099718. E-mail: marialaura.bolognesi@unibo.it.

ACKNOWLEDGMENTS

This research was supported by grants from MIUR (PRIN 2009_ESXPT2_001) and the University of Bologna, Italy. Crude **5** was a gift of Martine Fayolle (Université de Grenoble, France). Federica Lizzi, Federica Prati, and Birgit Hutter-Paier (JSW Lifesciences) are acknowledged for their excellent assistance. We thank Grace Fox for editing and proofreading the manuscript.

ABBREVIATIONS USED

Aβ, β-amyloid; AChE, acetylcholinesterase; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE-1, β-secretase; BuChE, butyrylcholinesterase; MTDL, multitarget-directed ligand; MW, molecular weight; NCE, new chemical entity; PAS, peripheral anionic site

REFERENCES

(1) Cavalli, A.; Bolognesi, M. L.; Capsoni, S.; Andrisano, V.; Bartolini, M.; Margotti, E.; Cattaneo, A.; Recanatini, M.; Melchiorre, C. A small molecule targeting the multifactorial nature of Alzheimer's disease. *Angew. Chem., Int. Ed.* **2007**, *46*, 3689−3692.

(2) Bolognesi, M. L.; Banzi, R.; Bartolini, M.; Cavalli, A.; Tarozzi, A.; Andrisano, V.; Minarini, A.; Rosini, M.; Tumiatti, V.; Bergamini, C.; Fato, R.; Lenaz, G.; Hrelia, P.; Cattaneo, A.; Recanatini, M.; Melchiorre, C. Novel class of quinone-bearing polyamines as multi-target-directed ligands to combat Alzheimer's disease. *J. Med. Chem.* **2007**, *50*, 4882−4897.

(3) Bolognesi, M. L.; Cavalli, A.; Melchiorre, C. Memoquin: a multi-target-directed ligand as an innovative therapeutic opportunity for Alzheimer's disease. *Neurotherapeutics* **2009**, *6*, 152−162.

(4) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.* **2008**, *51*, 347−372.

(5) Galdeano, C.; Viayna, E.; Arroyo, P.; Bidon-Chanal, A.; Blas, J. R.; Munoz-Torrero, D.; Luque, F. J. Structural determinants of the multifunctional profile of dual binding site acetylcholinesterase inhibitors as anti-Alzheimer agents. *Curr. Pharm. Des.* **2010**, *16*, 2818−2836.

(6) Geldenhuys, W. J.; Youdim, M. B.; Carroll, R. T.; Van der Schyf, C. J. The emergence of designed multiple ligands for neurodegenerative disorders. *Prog. Neurobiol.* **2011**, *94*, 347−359.

(7) Leon, R.; Garcia, A. G.; Marco-Contelles, J. Recent advances in the multitarget-directed ligands approach for the treatment of Alzheimer's disease. *Med. Res. Rev.* [Online early access]. DOI: 10.1002/med.20248. Published Online: Jul 26, **2011**.

(8) Mangialasche, F.; Solomon, A.; Winblad, B.; Mecocci, P.; Kivipelto, M. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol.* **2010**, *9*, 702−716.

(9) Portoghese, P. S. The role of concepts in structure−activity relationship studies of opioid ligands. *J. Med. Chem.* **1992**, *35*, 1927−1937, (Edward E. Smissman-Bristol-Myers Squibb Award Address).

(10) Decker, M. Homobivalent quinazolinimines as novel nanomolar inhibitors of cholinesterases with dirigible selectivity toward butyrylcholinesterase. *J. Med. Chem.* **2006**, *49*, 5411−5413.

(11) Rook, Y.; Schmidtke, K. U.; Gaube, F.; Schepmann, D.; Wunsch, B.; Heilmann, J.; Lehmann, J.; Winckler, T. Bivalent beta-carbolines as potential multitarget anti-Alzheimer agents. *J. Med. Chem.* **2010**, *53*, 3611−3617.

(12) Lenhart, J. A.; Ling, X.; Gandhi, R.; Guo, T. L.; Gerk, P. M.; Brunzell, D. H.; Zhang, S. "Clicked" bivalent ligands containing curcumin and cholesterol as multifunctional abeta oligomerization

inhibitors: design, synthesis, and biological characterization. *J. Med. Chem.* **2010**, *53*, 6198−6209.
(13) Pang, Y. P.; Quiram, P.; Jelacic, T.; Hong, F.; Brimijoin, S. Highly potent, selective, and low cost bis-tetrahydroaminacrine inhibitors of acetylcholinesterase. Steps toward novel drugs for treating Alzheimer's disease. *J. Biol. Chem.* **1996**, *271*, 23646−23649.
(14) Castro, A.; Martinez, A. Peripheral and dual binding site acetylcholinesterase inhibitors: implications in treatment of Alzheimer's disease. *Mini-Rev. Med. Chem.* **2001**, *1*, 267−272.
(15) Du, D. M.; Carlier, P. R. Development of bivalent acetylcholinesterase inhibitors as potential therapeutic drugs for Alzheimer's disease. *Curr. Pharm. Des.* **2004**, *10*, 3141−3156.
(16) Munoz-Torrero, D.; Camps, P. Dimeric and hybrid anti-Alzheimer drug candidates. *Curr. Med. Chem.* **2006**, *13*, 399−422.
(17) Haviv, H.; Wong, D. M.; Silman, I.; Sussman, J. L. Bivalent ligands derived from huperzine A as acetylcholinesterase inhibitors. *Curr. Top. Med. Chem.* **2007**, *7*, 375−387.
(18) Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Melchiorre, C. From dual binding site acetylcholinesterase inhibitors to multi-target-directed ligands (MTDLs): a step forward in the treatment of Alzheimer's disease. *Mini-Rev. Med. Chem.* **2008**, *8*, 960−967.
(19) Morphy, R.; Kay, C.; Rankovic, Z. From magic bullets to designed multiple ligands. *Drug Discovery Today* **2004**, *9*, 641−651.
(20) Bartolini, M.; Andrisano, V. Strategies for the inhibition of protein aggregation in human diseases. *ChemBioChem* **2010**, *11*, 1018−1035.
(21) Reinke, A. A.; Gestwicki, J. E. Insight into amyloid structure using chemical probes. *Chem. Biol. Drug Des.* **2011**, *77*, 399−411.
(22) Bolognesi, M. L.; Bartolini, M.; Mancini, F.; Chiriano, G.; Ceccarini, L.; Rosini, M.; Milelli, A.; Tumiatti, V.; Andrisano, V.; Melchiorre, C. Bis(7)-tacrine derivatives as multitarget-directed ligands: focus on anticholinesterase and antiamyloid activities. *ChemMedChem* **2010**, *5*, 1215−1220.
(23) Capsoni, S.; Andrisano, V.; Bartolini, M.; Bolognesi, M. L.; Cavalli, A.; Margotti, E.; Melchiorre, C.; Recanatini, M.; Cattaneo, A. S4-04-04 memoquin, a novel multifunctional compound for Alzheimer's disease: an up-date on preclinical studies. *Alzheimer's Dementia* **2006**, *2*, S73−S74.
(24) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2001**, *46*, 3−26.
(25) Forloni, G.; Colombo, L.; Girola, L.; Tagliavini, F.; Salmona, M. Anti-amyloidogenic activity of tetracyclines: studies in vitro. *FEBS Lett.* **2001**, *487*, 404−407.
(26) Bermejo-Bescos, P.; Martin-Aragon, S.; Jimenez-Aliaga, K. L.; Ortega, A.; Molina, M. T.; Buxaderas, E.; Orellana, G.; Csaky, A. G. In vitro antiamyloidogenic properties of 1,4-naphthoquinones. *Biochem. Biophys. Res. Commun.* **2010**, *400*, 169−174.
(27) Bolognesi, M. L.; Bartolini, M.; Tarozzi, A.; Morroni, F.; Lizzi, F.; Milelli, A.; Minarini, A.; Rosini, M.; Hrelia, P.; Andrisano, V.; Melchiorre, C. Multitargeted drugs discovery: balancing anti-amyloid and anticholinesterase capacity in a single chemical entity. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2655−2658.
(28) Bolognesi, M. L.; Calonghi, N.; Mangano, C.; Masotti, L.; Melchiorre, C. Parallel synthesis and cytotoxicity evaluation of a polyamine−quinone conjugates library. *J. Med. Chem.* **2008**, *51*, 5463−5467.
(29) Fryatt, T.; Pettersson, H. I.; Gardipee, W. T.; Bray, K. C.; Green, S. J.; Slawin, A. M. Z.; Beall, H. D.; Moody, C. J. Novel quinolinequinone antitumor agents: structure−metabolism studies with NAD(P)H:quinone oxidoreductase (NQO1). *Bioorg. Med. Chem.* **2004**, *12*, 1667−1687.
(30) Bolognesi, M. L.; Cavalli, A.; Bergamini, C.; Fato, R.; Lenaz, G.; Rosini, M.; Bartolini, M.; Andrisano, V.; Melchiorre, C. Toward a rational design of multitarget-directed antioxidants: merging memoquin and lipoic acid molecular frameworks. *J. Med. Chem.* **2009**, *52*, 7883−7886.
(31) Inestrosa, N. C.; Alvarez, A.; Perez, C. A.; Moreno, R. D.; Vicente, M.; Linker, C.; Casanueva, O. I.; Soto, C.; Garrido, J. Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. *Neuron* **1996**, *16*, 881−891.
(32) Bartolini, M.; Bertucci, C.; Cavrini, V.; Andrisano, V. beta-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. *Biochem. Pharmacol.* **2003**, *65*, 407−416.
(33) Appleyard, M. E.; McDonald, B. Acetylcholinesterase and butyrylcholinesterase activities in cerebrospinal fluid from different levels of the neuraxis of patients with dementia of the Alzheimer type. *J. Neurol. Neurosurg. Psychiatry* **1992**, *55*, 1074−1078.
(34) Bartolini, M.; Bertucci, C.; Bolognesi, M. L.; Cavalli, A.; Melchiorre, C.; Andrisano, V. Insight into the kinetic of amyloid beta (1−42) peptide self-aggregation: elucidation of inhibitors' mechanism of action. *ChemBioChem* **2007**, *8*, 2152−2161.
(35) Reinke, A. A.; Ung, P. M. U.; Quintero, J. J.; Carlson, H. A.; Gestwicki, J. E. Chemical probes that selectively recognize the earliest Abeta oligomers in complex mixtures. *J. Am. Chem. Soc.* **2010**, *132*, 17655−17657.
(36) Convertino, M.; Pellarin, R.; Catto, M.; Carotti, A.; Caflisch, A. 9,10-Anthraquinone hinders beta-aggregation: hHow does a small molecule interfere with Abeta-peptide amyloid fibrillation? *Protein Sci.* **2009**, *18*, 792−800.
(37) Scherzer-Attali, R.; Pellarin, R.; Convertino, M.; Frydman-Marom, A.; Egoz-Matia, N.; Peled, S.; Levy-Sakin, M.; Shalev, D. E.; Caflisch, A.; Gazit, E.; Segal, D. Complete phenotypic recovery of an Alzheimer's disease model by a quinone−tryptophan hybrid aggregation inhibitor. *PLoS One* **2010**, *5*, e11101.
(38) Ortega, A.; Rincon, A.; Jimenez-Aliaga, K. L.; Bermejo-Bescos, P.; Martin-Aragon, S.; Molina, M. T.; Csaky, A. G. Synthesis and evaluation of arylquinones as BACE1 inhibitors, beta-amyloid peptide aggregation inhibitors, and destabilizers of preformed beta-amyloid fibrils. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2183−2187.
(39) Turk, B. Targeting proteases: successes, failures and future prospects. *Nat. Rev. Drug Discovery* **2006**, *5*, 785−799.
(40) Czvitkovich, S.; Duller, S.; Mathiesen, E.; Lorenzoni, K.; Imbimbo, B. P.; Hutter-Paier, B.; Windisch, M.; Wronski, R. Comparison of pharmacological modulation of APP metabolism in primary chicken telencephalic neurons and in a human neuroglioma cell line. *J. Mol. Neurosci.* **2011**, *43*, 257−267.
(41) Morphy, R.; Rankovic, Z. Fragments, network biology and designing multiple ligands. *Drug Discovery Today* **2007**, *12*, 156−160.
(42) Rizzo, S.; Riviere, C.; Piazzi, L.; Bisi, A.; Gobbi, S.; Bartolini, M.; Andrisano, V.; Morroni, F.; Tarozzi, A.; Monti, J. P.; Rampa, A. Benzofuran-based hybrid compounds for the inhibition of cholinesterase activity, beta amyloid aggregation, and abeta neurotoxicity. *J. Med. Chem.* **2008**, *51*, 2883−2886.