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Quinones bearing non-steroidal anti-inflammatory fragments as multitarget ligands for Alzheimer's disease

Federica Prati [a,b], Manuela Bartolini [a], Elena Simoni [a], Angela De Simone [c], Antonella Pinto [d], Vincenza Andrisano [c], Maria Laura Bolognesi [a,*]

[a] Department of Pharmacy and Biotechnology, Alma Mater Studiorum University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[b] Department of Drug Discovery and Development, Italian Institute of Technology, Via Morego 30, 16163 Genoa, Italy
[c] Department for Life Quality Studies, Alma Mater Studiorum University of Bologna, Corso D'Augusto 237, 47921 Rimini, Italy
[d] Department of Drug Sciences—Pharmacology, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

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ABSTRACT

The anti-amyloid properties shared by several quinones inspired the design of a new series of hybrids derived from the multi-target drug candidate memoquin (**1**). The hybrids consist of a central benzoquinone core and a fragment taken from non-steroidal anti-inflammatory drugs, connected through polyamine linkers. The new hybrids retain the potent anti-aggregating activity of the parent **1**, while exhibiting micromolar AChE inhibitory activities. Remarkably, **2**, **4**, (*R*)-**6** and (*S*)-**6** were Aβ aggregation inhibitors even more potent than **1**. The balanced amyloid/cholinesterase inhibitory profile is an added value that makes the present series of compounds promising leads against Alzheimer's disease.

Alzheimer's disease (AD) is an incurable neurodegenerative malady of the central nervous system with a complex multifactorial etiology.[1,2] In response to such molecular complexity, a polypharmacological drug discovery approach is envisaged to provide therapeutic benefits where currently available single-target drugs have failed. There are two main ways to achieve polypharmacology: drug combinations and single chemical entities that have multiple biological properties, that is the so called multitarget drugs. It has been advanced that the use of multitarget drugs could have general inherent advantages over combination therapies. Administering one compound with multiple biological actions guarantees the simultaneous presence of the molecule in those districts of the body, where the active principle needs to work and interact with its multiple targets. Focusing on AD, two critical issues are addressed: (i) the risk of possible drug–drug interactions in elderly patients would be reduced and (ii) the therapeutic regimen greatly simplified, with the prospect of enhanced patient compliance.[3] Exploiting a multitarget drug discovery approach, memoquin (**1**; Fig. 1) was developed as one of the first multitarget drug candidate against AD.[4] Compound **1** is a free-radical scavenger and an inhibitor of amyloid-β (Aβ) aggregation and acetylcholinesterase (AChE) activity. In vivo, **1** acts as a cognitive enhancer in several AD mouse models, strengthening the value of a multitarget strategy in AD.[5,6]

From a structural point of view, **1** is a hybrid molecule obtained by integrating a benzoquinone core into a polyamine chain.[7] The resulting 2,5-diamino-benzoquinone scaffold of **1** has been deemed to have a crucial role in conferring the multiple activities. In particular, thanks to the planar and aromatic features and the hydrogen bonding capability, it might be essential in modulating protein–protein interactions involved in AD pathogenesis.[8] In fact, several other quinones (either benzo-, naphtho- or anthraquinones) have been shown to effectively inhibit the aggregation of various amyloidogenic proteins.[9–13]

The anti-aggregating capability we could verify for several hybrid molecules featuring a 2,5-diamino-1,4-benzoquinone core connecting two aromatic appending moieties, lends further support to this hypothesis.[14–18] The selected aromatic moieties where taken from known amyloidophilic agents, such as, among others, curcumin, benzofurans and benzothiazoles. Intriguingly, such molecules were shown to be effective inhibitors of Aβ fibril formation,[14–16] but also of prion protein aggregation.[17,18] For these reasons, the 2,5-diamino-1,4-benzoquinone fragment can be considered as a truly privileged motif to interfere with protein–protein interactions and a useful starting point for the design of novel multitarget ligands against AD. Expanding this basic idea, we developed herein a further series of quinone-based hybrids. Looking for novel aromatic fragments to be appended to

* Corresponding author.
E-mail address: marialaura.bolognesi@unibo.it (M.L. Bolognesi).

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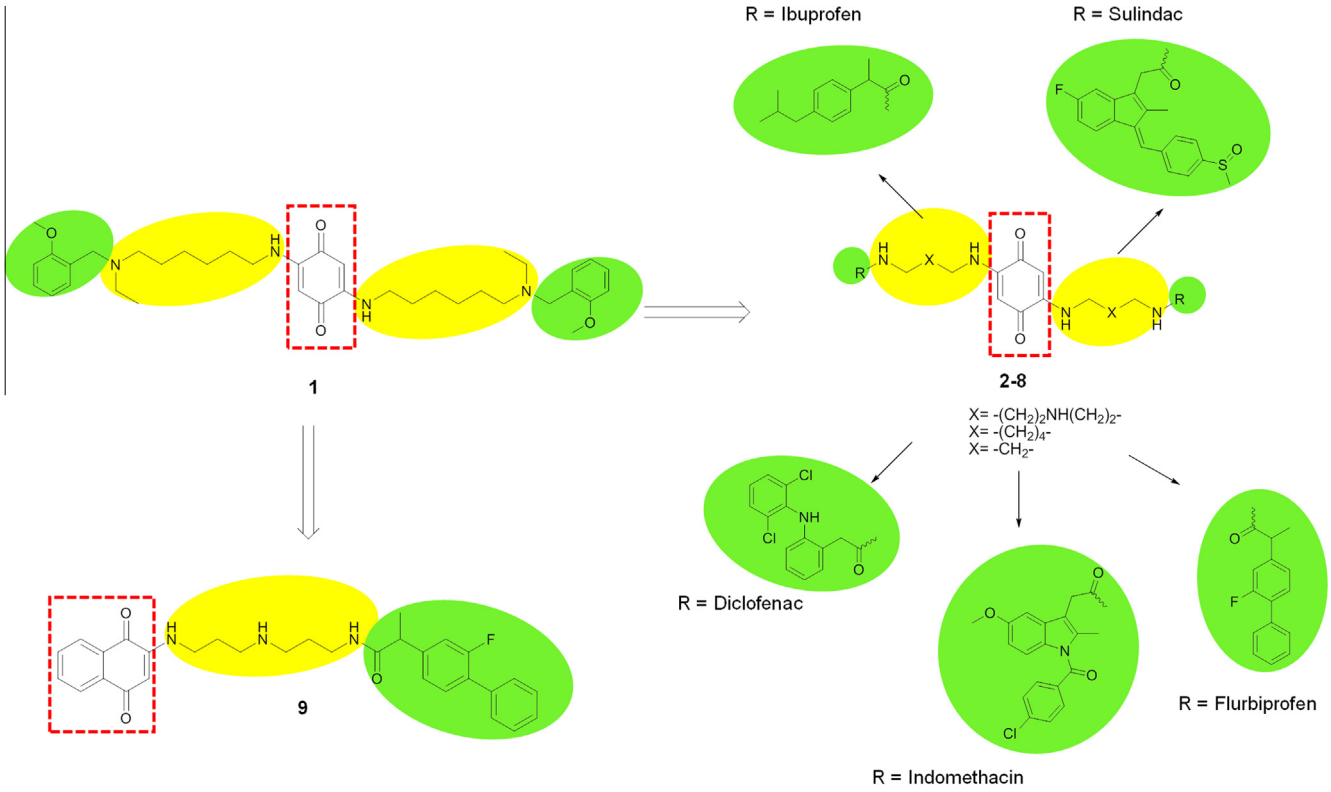


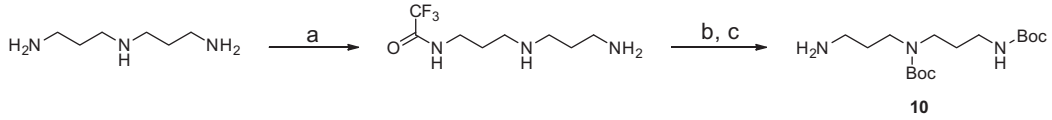
Figure 1. Design strategy for compounds **2**–**9**.

the benzoquinone core, we turned our attention to non-steroidal anti-inflammatory drugs (NSAIDs), that is ibuprofen, sulindac, indomethacin, diclofenac and flurbiprofen (Fig. 1). This choice was motivated by the fact that this specific NSAID subset has been demonstrated to directly inhibit Aβ fibril formation and destabilize preformed Aβ fibrils in vitro.[19] In addition, all of them have also been shown to affect the production of Aβ.[20] As a further positive evidence, epidemiological studies indicated that NSAIDs might lower the risk of developing AD.[21] Thus, hybrids **2**–**9** were designed and synthesized by connecting the 2,5-diamino-benzoquinone core with the NSAID-pharmacophoric elements through the polyamine linkers that were successfully exploited in a previous endorsement.[18] Considering that monomeric naphthoquinone analogues of **1** retained the multitarget profile of the parent compound,[22] the monomeric hybrid **9** was also designed (Fig. 1).

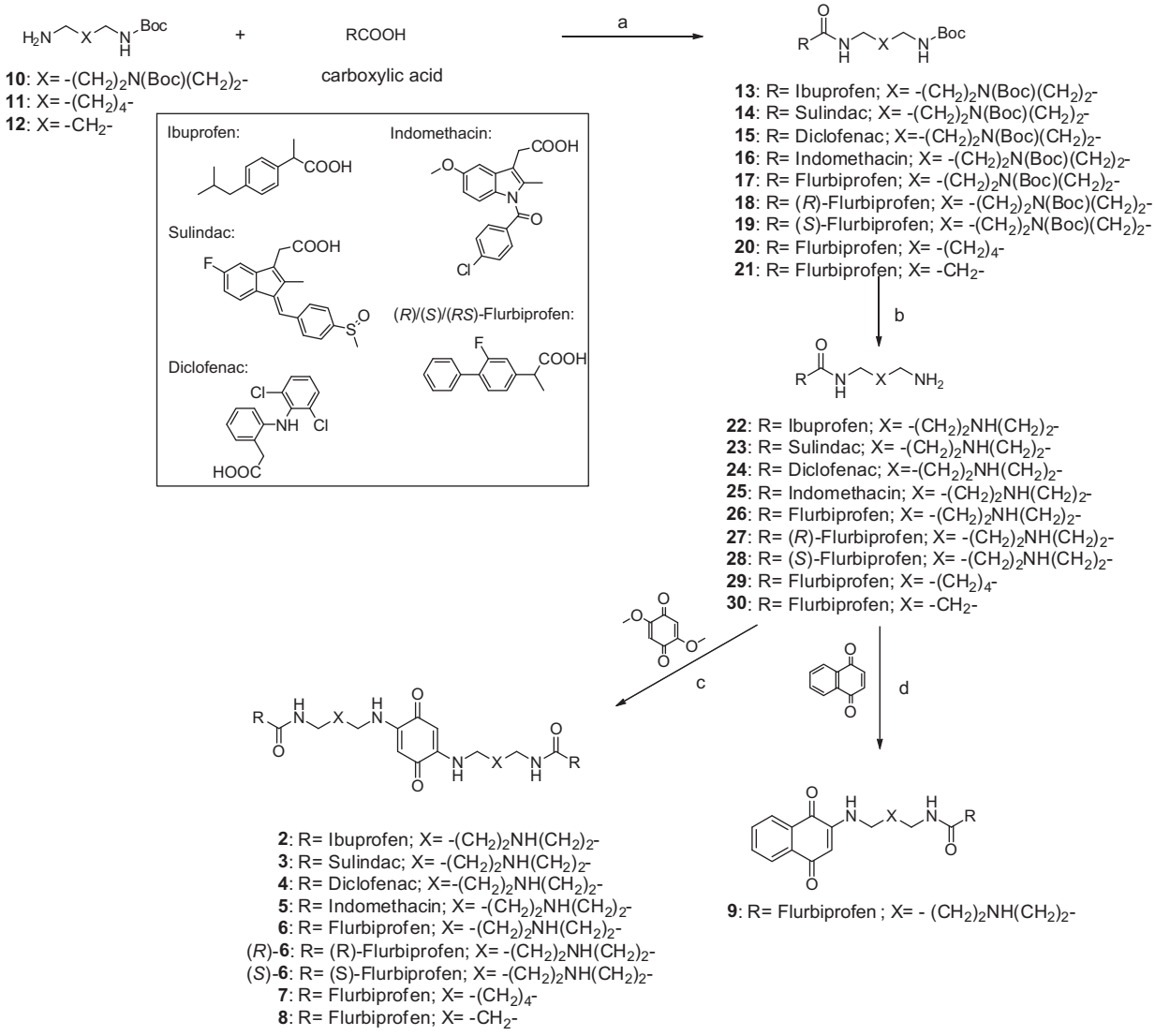
The synthetic route used to access the target compounds is summarized in Schemes 1 and 2.[23] The presence in all the selected NSAIDs of a free carboxylic acid functionality provided a straightforward attachment point to the amino group of the polyamine linker through amide formation. Thus, the properly Boc-protected polyamines **10**–**12**[24] were coupled with the respective NSAID fragments through standard coupling conditions, affording intermediates **13**–**21**. After a deprotection step, the primary amino groups of **22**–**30** were connected to the central benzoquinone core by exploiting an efficient substitution reaction on 2,5-dimethoxy-benzoquinone.[7] In the case of **9**, the amino group of **26** was connected to the naphthoquinone moiety through a Michael addition reaction.

To characterize the in vitro anti-AD multitarget profile of our derivatives, the ability to reduce $A\beta_{42}$ spontaneous aggregation and secretion (through the inhibition of beta-secretase activity) and the inhibitory activity on the cholinesterase enzymes that is, human AChE and butyrylcholinesterase (BuChE), were investigated in comparison to **1**.

Even if the main triggering event leading to the AD pathology is still under debate, Aβ aggregation is well recognized to be connected to AD pathogenesis and progression. Therefore, it is still feasible that anti-amyloid agents might impact the disease progression in a meaningful way.[25,26] Thus, the in vitro anti-amyloid potential of all synthesized quinone-NSAID hybrids was determined using a Thioflavin T (ThT)-based fluorometric assay.[8] Inhibition studies were carried out by incubating $A\beta_{42}$ (50 μM) with and without a five fold lower concentration of tested compound and by evaluating the decrease in ThT fluorescence intensity at 490 nm (λ_{exc} = 446 nm). We were pleased to verify that all hybrids carrying a triamine linker (**2**–**6**) exhibited percentages of inhibition ranging from 55% to 90% (Table 1). In agreement with the anti-aggregating properties reported for some NSAIDs,[19] the presence of a flurbiprofen (**6**), ibuprofen (**2**), indomethacin (**5**), or diclofenac (**4**) fragment leads to an inhibitory potency slightly higher than that of the parent compound **1**. On the basis of these promising results, the correspondent IC_{50} values were determined. In addition, the high inhibitory potency (% inhibition >90%) shown by **6** as racemic mixture prompted us to investigate the activity of the single enantiomers ((*R*)-**6** and (*S*)-**6**). The effect of the



Scheme 1. Reagents and conditions: (a) $EtOCOCF_3$, MeOH, −50 °C; (b) $(Boc)_2O$; (c); $NaOH/H_2O$.



Scheme 2. Reagents and conditions: (a) THF, EDC, Et_3N; (b) TFA; (c) 0.5 equiv 2,5-dimethoxybenzoquinone, EtOH, 50–80 °C for 3–5 h; (d) 1 equiv naphthoquinone, MeOH, rt, overnight.

Table 1
Inhibitory activity on amyloid self-aggregation and human AChE and BuChE activities by **2**–**9** and reference compound **1**[a]

	R	X	Inhibition of $A\beta_{42}$ aggregation		Inhibition of cholinesterase activity	
			[*I*] = 10 μM[a] (%)	IC_{50}[b] (μM)	hAChE IC_{50}[c] (μM)	hBuChE IC_{50}[c] (μM)
1			66.8 ± 4.4	5.93	$(1.55 \pm 0.11)10^{-3}$	1.44 ± 0.10
2	Ibuprofen	$-(CH_2)_2NH-(CH_2)_2-$	82.6 ± 4.5	4.83	0.60 ± 0.02	22.5 ± 1.5
3	Sulindac	$-(CH_2)_2NH-(CH_2)_2-$	55.0 ± 3.0	8.18	9.54 ± 0.29	14.7 ± 0.2
4	Diclofenac	$-(CH_2)_2NH-(CH_2)_2-$	74.9 ± 0.2	3.15	15.4 ± 1.1	0.36 ± 0.02
5	Indomethacin	$-(CH_2)_2NH-(CH_2)_2-$	80.1 ± 3.0	6.11	10.3 ± 3.7	18.7 ± 1.0
6	Flurbiprofen	$-(CH_2)_2NH-(CH_2)_2-$	>90	—	—	—
(*R*)-**6**	(*R*)-Flurbiprofen	$--(CH_2)_2NH-(CH_2)_2-$	87.2 ± 1.6	4.77	27.8 ± 1.1	9.98 ± 0.60
(*S*)-**6**	(*S*)-Flurbiprofen	$-(CH_2)_2NH-(CH_2)_2-$	>90	4.43	2.89 ± 0.12	7.77 ± 0.21
7	Flurbiprofen	$-(CH_2)_4-$	nd[d]	nd[d]	>20	>20
8	Flurbiprofen	$-CH_2-$	12.7 ± 3.8	nd	>20	116 ± 2
9	See Figure 1 for structure		76.4 ± 2.5	6.88	107 ± 7	16.4 ± 0.2
	Tacrine		na[e]	—	0.383 ± 0.02	0.058 ± 0.005

[a] Percent inhibition of 50 μM $A\beta_{42}$ aggregation by 10 μM compound. The $A\beta_{42}$/inhibitor ratio was equal to 5:1.
[b] IC_{50} represents the concentration of inhibitor required to decrease the ThT fluorescence intensity at 490 nm by 50%.
[c] Human recombinant AChE and BuChE from human serum were used. IC_{50} values, determined by using Ellman's method,[29] represent the concentration of inhibitor required to decrease enzyme activity by 50%.
[d] nd = not determined. Not determined because not soluble in the assay conditions.
[e] % Inhibition <5% at 50 μM. na = not active.

polyamine linker was also evaluated, by synthesizing the 1,3-hexane- (**7**) and 1,6-propane-diamino (**8**) congeners of **6**.

All the tested hybrids showed low micromolar activities that make them potent anti-aggregating agents. Differences in the inhibitory potencies are quite little within the series, being the difference between the less and the most active within two folds.

No enantioselectivity was found for **6**, as (*R*)-**6** and (*S*)-**6** display very similar inhibitory activities on amyloid aggregation. Conversely, the depletion in the polyamine linker of **6** of the protonable nitrogen atom not only influenced the physical–chemical properties, but also modified its ability of interacting with amyloid oligomers and fibrils. In fact, **7** could be not tested because not soluble in the assay conditions, whereas **8** was a very weak inhibitor with a percentage of inhibition of only 12%.

Notably, **4** showed a remarkable IC_{50} of 3.15 μM, which makes it the most active of the current series, but also the top-ranked among all **1**'s derivatives tested so far.[7,15,16,22]

As a general comment, the significant anti-aggregating activity shown by **2**–**8**, which all share a common bivalent structure, reinforces the intriguing hypothesis that such palindromic compounds could cross-link two amyloid molecules and consequently effectively perturb the fibrillogenesis process.[27]

The anti-fibrillogenic activities showed by naphthoquinone **9** is also intriguing. Indeed, despite the lack of a bivalent structure, **9** shows an IC_{50} value which is slightly higher than that of **1** (6.33 μM vs 5.88 μM, respectively). This result is in line with the outstanding anti-amyloid profile shown by the 1,4-naphthoquinon-2-yl-L-tryptophan developed by Gazit and coworkers.[13]

To expand the anti-amyloid profile of **2**–**9**, their ability to inhibit the amyloidogenic activity of human recombinant beta-secretase-1 (BACE-1) enzyme was also investigated. When screened at a concentration of 3 μM using M-2420 (Bachem) as assay substrate, these compounds showed to be weak BACE-1 inhibitors with percentages of inhibition ranging from 20% to 30%. Due to the low % of inhibition obtained at the screening concentration, IC_{50} values were not determined.

To assess safety profile, the in vitro cytotoxicity of the most interesting derivatives, namely compounds **4**, (*R*)- and (*S*)-**6** and **9**, was evaluated using HEK 293 cell line. Results showed that all the tested compounds, but (*S*)-**6**, did not significantly reduce cell viability up to 10 μM (Fig. 2).

Building on the low in vitro cytotoxicity shown by **9** in HEK cell line (Fig. 2) and the good cell toxicity profile on primary neurons shown by similar naphtoquinone derivatives,[22] the effect of **9** on APP processing was evaluated in a cellular context. The study was carried out in embryonic chicken telencephalon neurons to assess the effect on Aβ secretion.[22] All data were corrected with mean neurons viability evaluated in the MTT assay. A mild reduction on $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ secretion was observed at 10 μM, but, because of some detrimental toxicity displayed at 25 μM and 50 μM, a clear concentration-dependent decrease in Aβ secretion could not be observed (Fig. 3).

From the clinical use it is well known that AChE inhibitors, albeit palliative, are effective in improving activities of daily living, behavior, and slowing cognitive decline in moderate to severe AD patients. Moreover, APP processing is under the control of several major neurotransmitters such as acetylcholine (ACh), therefore an ACh level increase by AChE inhibitors can also lower amyloid production.[28] Thus, the anticholinesterase activity can still be considered an important component in the overall multitarget profile of an AD drug candidate. On this basis, the anticholinesterase activities of **2**–**9** were also evaluated (Table 1) in comparison with **1**. An analysis of the results revealed that the new hybrids still keep inhibitory potencies against human AChE (hAChE) and human BuChE (hBuChE), in the micromolar range. However, as expected on the basis of previous results,[7] they are three fold less potent than **1**, which is a nanomolar AChE inhibitor. It is highly feasible that the lack of a protonable nitrogen atom (interacting with the AChE anionic site) in the case of **7** and **8**, or of the *N*-ethyl

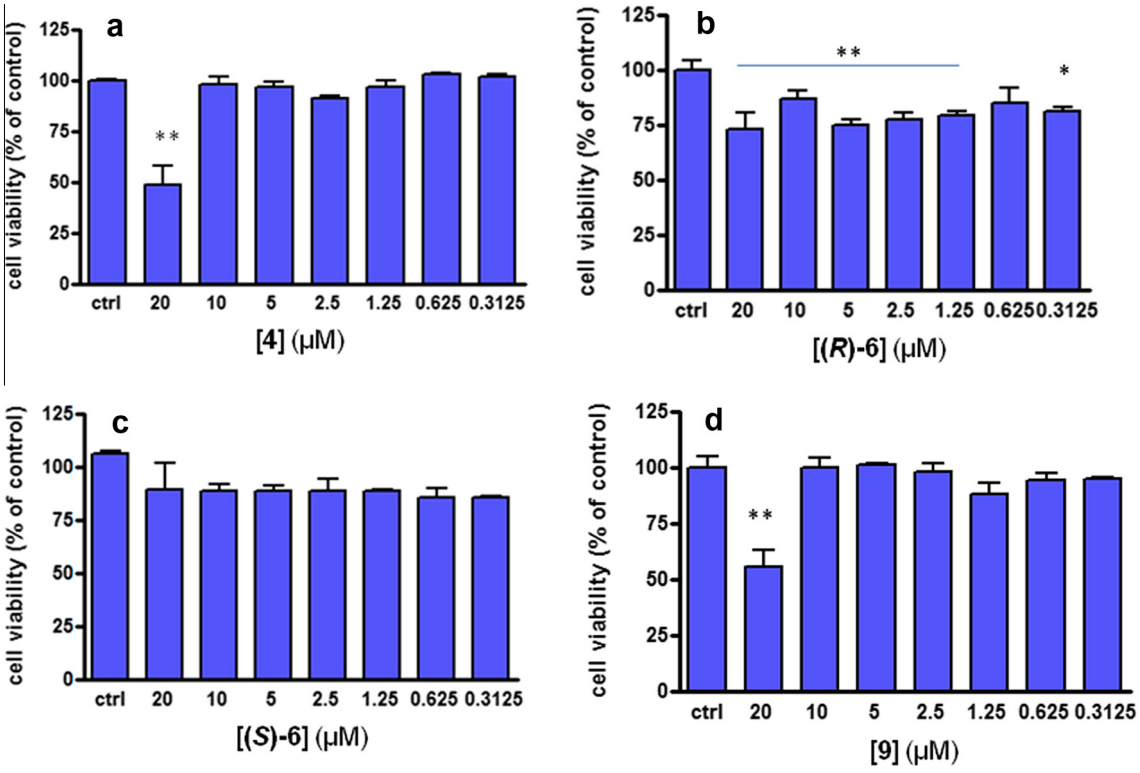


Figure 2. MTT assay to measure cell viability in HEK 293 cell line after treatment with **4** (a), (*R*)-**6** (b), (*S*)-**6** (c), and **9** (d) at concentrations ranging from 0.3125 to 20 μM. Ctrl: control. Statistical analysis was performed with Dunnett's multiple comparison test with $^{*}p$ <0.05 $^{**}p$ <0.01 versus untreated cells.

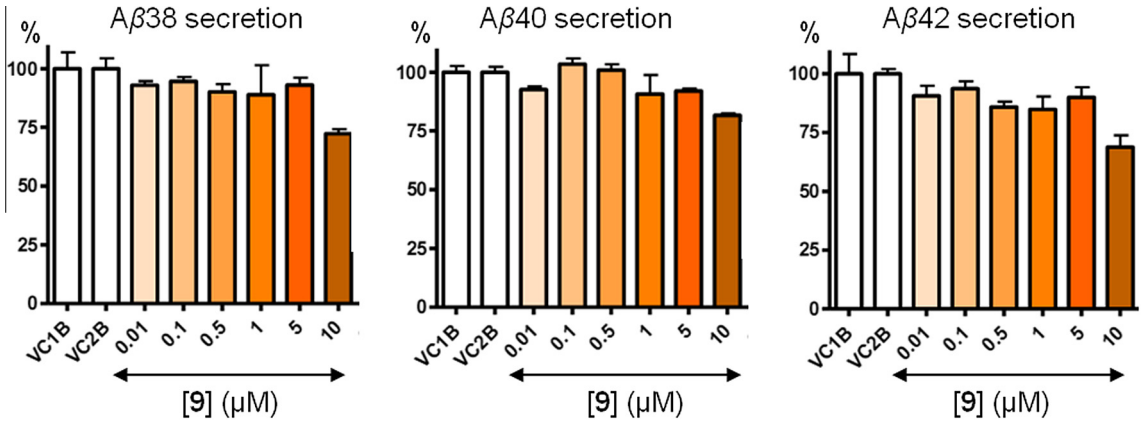


Figure 3. Secretion of $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ after treatment with **9**. Values represent the mean and the SEM of $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ secretion in percent from one experiment performed in 24-well plates ($n = 3$ per experiment) for each inhibitor concentration and for controls. Since cells were cultured in two 24-well plates, each plate contained a separate vehicle control (VC) group. VC1B indicates the vehicle control on plate 1 and VC2B on plate 2. Vehicle controls were set as 100%.

substituent for **2**–**6** can account for that.[7] Among the homogenous triamine series **2**–**6**, the most active is the ibuprofen derivative **2** (IC_{50} = 0.60 μM), which turned out more potent than the marketed drugs galantamine (IC_{50} = 2.01 μM)[7] and rivastigmine (3.03 μM).[7] A slightly lower activity is observed for (*S*)-**6**, whereas the enantiomer (*R*)-**6** is ten times less effective. Compound **9**, carrying the flurbiprofen fragment, but devoid of a bivalent structure, resulted less potent than (*R*)-**6** and (*S*)-**6**. Moreover, the type of NSAID fragment seems to influence the selectivity for one of the two ChEs. Indeed while **2** (bearing the ibuprofen fragment) is 37.5 times more potent on AChE, the derivative **4** (bearing the diclofenac fragment) is 42.8 more potent on BuChE. To confirm the importance of a proper integration of single fragments into a new chemical entity, an equimolar mixture of the three fragments composing **2** (bis-(3-aminopropyl)amine, 1,4-benzoquinone and ibuprofen) was assayed. At 67 μM, inhibition resulted 26.3% ± 1.8% and <10% on hAChE and hBuChE, respectively whereas, at the same concentration, compound **2** gave a complete inhibition of hAChE activity and ~70% inhibition of the hBuChE's hydrolyzing activity. These results further confirm that the suitable incorporation of the different structural elements into a new single chemical entity enables the achievement of higher inhibitory potency and the selectivity.

Taken together, the results presented here indicate that targeting Aβ by a quinone structure is a promising approach for the inhibition of amyloid fibrillogenesis. Because Aβ misfolding and aggregation are still considered key early pathogenic events in AD, it is of great interest for a potential disease-modifying treatment.

Additionally, **2**–**9** have been found to display another pharmacological effect, namely a cholinesterase inhibitory activity, which should nicely complement the anti-amyloid one.

In conclusion, thanks to a balanced micromolar Aβ/cholinesterase profile, the here presented quinone–NSAID hybrids could be a promising starting point in the search for new multitarget ligands against AD.

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References and notes

1. Montine, T. J.; Larson, E. B. *JAMA* **2009**, *302*, 2593.
2. Querfurth, H. W.; LaFerla, F. M. N. *Engl. J. Med.* **2010**, *362*, 329.
3. Bolognesi, M. L. *Curr. Med. Chem.* **2013**, *20*, 1639.
4. Cavalli, A.; Bolognesi, M. L.; Capsoni, S.; Andrisano, V.; Bartolini, M.; Margotti, E.; Cattaneo, A.; Recanatini, M.; Melchiorre, C. *Angew. Chem., Int. Ed.* **2007**, *46*, 3689.
5. Bolognesi, M. L.; Cavalli, A.; Melchiorre, C. *Neurotherapeutics* **2009**, *6*, 152.
6. Capurro, V.; Busquet, P.; Lopes, J. P.; Bertorelli, R.; Tarozzo, G.; Bolognesi, M. L.; Piomelli, D.; Reggiani, A.; Cavalli, A. *PLoS One* **2013**, *8*, e56870.
7. 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. *J. Med. Chem.* **2007**, *50*, 4882.
8. Bartolini, M.; Bertucci, C.; Bolognesi, M. L.; Cavalli, A.; Melchiorre, C.; Andrisano, V. *ChemBioChem* **2007**, *8*, 2152.
9. Ono, K.; Hasegawa, K.; Naiki, H.; Yamada, M. *Biochem. Biophys. Res. Commun.* **2005**, *330*, 111.
10. Convertino, M.; Pellarin, R.; Catto, M.; Carotti, A.; Caflisch, A. *Protein Sci.* **2009**, *18*, 792.
11. Kim, Y.; Lee, J. H.; Ryu, J.; Kim, D. J. *Curr. Pharm. Des.* **2009**, *15*, 637.
12. Bermejo-Bescos, P.; Martin-Aragon, S.; Jimenez-Aliaga, K. L.; Ortega, A.; Molina, M. T.; Buxaderas, E.; Orellana, G.; Csaky, A. G. *Biochem. Biophys. Res. Commun.* **2010**, *400*, 169.
13. 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. *PLoS One* **2010**, *5*, e11101.
14. Bolognesi, M. L.; Cavalli, A.; Bergamini, C.; Fato, R.; Lenaz, G.; Rosini, M.; Bartolini, M.; Andrisano, V.; Melchiorre, C. *J. Med. Chem.* **2009**, *52*, 7883.
15. Bolognesi, M. L.; Bartolini, M.; Rosini, M.; Andrisano, V.; Melchiorre, C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4312.
16. Bolognesi, M. L.; Bartolini, M.; Tarozzi, A.; Morroni, F.; Lizzi, F.; Milelli, A.; Minarini, A.; Rosini, M.; Hrelia, P.; Andrisano, V.; Melchiorre, C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2655.
17. Tran, H. N.; Bongarzone, S.; Carloni, P.; Legname, G.; Bolognesi, M. L. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1866.
18. Bongarzone, S.; Tran, H. N.; Cavalli, A.; Roberti, M.; Carloni, P.; Legname, G.; Bolognesi, M. L. *J. Med. Chem.* **2010**, *53*, 8197.
19. Hirohata, M.; Ono, K.; Naiki, H.; Yamada, M. *Neuropharmacology* **2005**, *49*, 1088.
20. Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrzik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Bulter, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. *Nature* **2001**, *414*, 212.
21. in t' Veld, B. A.; Ruitenberg, A.; Hofman, A.; Launer, L. J.; van Duijn, C. M.; Stijnen, T.; Breteler, M. M.; Stricker, B. H. *N. Engl. J. Med.* **2001**, *345*, 1515.
22. Bolognesi, M. L.; Chiriano, G.; Bartolini, M.; Mancini, F.; Bottegoni, G.; Maestri, V.; Czvitkovich, S.; Windisch, M.; Cavalli, A.; Minarini, A.; Rosini, M.; Tumiatti, V.; Andrisano, V.; Melchiorre, C. *J. Med. Chem.* **2011**, *54*, 8299.
23. Spectra data for compound **4**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.68–1.80 (m, 8H), 2.61–2.73 (m, 8H), 3.26–3.30 (m, 4H), 3.42–3.50 (m, 4H), 3.51 (s, 2H exchangeable with D_2O), 3.85 (s, 4H), 5.35 (s, 2H), 6.51 (d, J = 8.0, 2H), 6.83–7.18 (m complex, 8H), 7.18–7.37 (m, 4H+2H exchangeable with D_2O), 7.76 (br s, 2H exchangeable with D_2O), 8.48 (br s, 2H exchangeable with D_2O); ESI–MS (m/z): 923 ($M+H^+$), 945 ($M+Na^+$).
24. Bolognesi, M. L.; Calonghi, N.; Mangano, C.; Masotti, L.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 5463.
25. Bartolini, M.; Andrisano, V. *ChemBioChem* **2010**, *11*, 1018.
26. Belluti, F.; Rampa, A.; Gobbi, S.; Bisi, A. *Expert Opin. Ther. Pat.* **2013**, *23*, 581.
27. Kolstoe, S. E.; Mangione, P. P.; Bellotti, V.; Taylor, G. W.; Tennent, G. A.; Deroo, S.; Morrison, A. J.; Cobb, A. J.; Coyne, A.; McCammon, M. G.; Warner, T. D.; Mitchell, J.; Gill, R.; Smith, M. D.; Ley, S. V.; Robinson, C. V.; Wood, S. P.; Pepys, M. B. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 20483.
28. Fisher, A. *J. Neurochem.* **2012**, *120*, 22.
29. Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Feather-Stone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.