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Synthesis and evaluation of a library of 2,5-bisdiamino-benzoquinone derivatives as probes to modulate protein–protein interactions in prions

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ABSTRACT

A small library combining two different benzoquinone cores with seven (L) amino acid methyl esters (alanine, Nω-nitro-arginine, Nε-BOC-lysine, isoleucine, methionine, phenylalanine and tryptophan) was prepared and tested for prion replication inhibition in ScGT1 cells. The most potent hit, 6a, displayed an EC_{50} value of 0.87 μM, which is very close to that of quinacrine (0.4 μM).

Protein–protein interactions (PPIs) are crucial elements in mediating diverse cellular physiological and pathological events.[1] They are involved in fibrillation processes and thus play a pivotal role in the pathogenesis of several neurodegenerative diseases.[2,3]

Systematic analysis of PPI interfaces reveals great heterogeneity, from large and flat to narrow and structured interactions.[4] However, the majority of PPIs deal with protein surfaces,[5] where a complex network of weak interactions takes place.

Peptides may be good PPI blockers.[6,7] However, they are not optimal drug candidates, due to problems with bioavailability and enzymatic degradation. To overcome this limitation, one could use combinatorial chemical libraries based on small molecules. However, the widely spaced interactions required for PPI blockers are difficult to mimic with small molecules. Despite this challenge, the strategy holds great potential for identifying novel lead compounds against PPIs.[8] The extreme attractiveness of PPIs as drug targets has led to important progresses in this field in the last decade.[1,9,10] In particular, Janda and co-workers have recently demonstrated the ability of what they have named "credit card" libraries to disrupt PPIs of biological relevance.[11] The chemical structures of these libraries are built upon flat, rigid scaffolds, decorated with appended groups that span a wide range of size, aromaticity, polarity, and hydrogen-bonding capability.[11] Their rationale was based on the concept that the 'hot spot' regions in protein–protein interfaces are rich in aromatic residues.

Here, we focus on PPIs in prion diseases. These diseases are characterized by the aggregation and accumulation of a misfolded prion protein, PrP^{Sc}, which derives from a post-translational conformational change of the host-encoded cellular prion protein, PrP^{C}.[12] The conformational transition process involved remains enigmatic. However, regardless of the initiating event, PrP^{Sc} appears to act as a conformational template by which PrP^{C} is converted to a new molecule of PrP^{Sc}, through PPIs.[12]

So far, several peptides have been developed with the specific aim of blocking PPIs and reversing the aberrant conformational changes. A short synthetic peptide (iPrP13, DAPAAPAGPAVPV), designed by Soto and co-workers on the basis of sequence homology with PrP^{C}, acted as a β-sheet breaker, inducing unfolding of β-pleated sheet structure.[13] More recently, Gilbert and co-workers[14] reported on a series of small peptides active at levels of 100 μM in two prion disease models and in an in vitro anti-aggregation polymerization assay. Prompted by the advantages of using small molecules as PPI inhibitors as opposed to peptides, here we propose the planar 2,5-bisdiamino-benzoquinone scaffold as a privileged motif in modulating PPIs. This is based on (i) Janda's criteria for credit card libraries[11]; (ii) the finding that a 2,5-bisdiamino-benzoquinone derivative binds to β-amyloid (Aβ), and interferes with the native ability of Aβ to self-assemble, by disrupting PPIs.[15] Due to a resonance effect, a hydrophobic and planar system is generated in 2,5-bisdiamino-benzoquinones. This should, in principle, perturb

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PPIs in the fibrillogenesis processes.[16] Therefore, in our search for novel anti-prion compounds, we decided to attach seven amino acids methyl esters to two different benzoquinone cores, generating a small combinatorial library of fourteen 2,5-bisdiamino-benzoquinones (**1–7a** and **1–7b**), reported in Figure 1. The selected amino acid esters (AlaOMe (**1**), Nω-Nitro-ArgOMe (**2**), Nε-BOC-LysOMe (**3**), IleOMe (**4**), MetOMe (**5**), PheOMe (**6**), TrpOMe (**7**)) act as capping groups, allowing us to enlarge the library's chemical diversity by exploiting differences in size, aromaticity, polarity, and hydrogen-bonding capability. Analysis of natural amino acids involved in PPIs revealed that Trp, Phe, Tyr, and Ile are the most important in driving aggregation.[17] Consequently, it is highly conceivable that the novel derivatives bearing these motifs might compete for binding and, therefore, efficiently disrupt the assembly of prion protein.

To develop an efficient parallel synthesis approach, we focused on the displacement reaction of tetrahalo-substituted quinones (chloranil, **a** and bromanil, **b**) with amino acid methyl esters (**1–7**) to afford a library of fourteen 2,5-diamino-3,6-dihalo-[1,4]benzoquinone derivatives. We carried out a one-pot reaction at room temperature that, in most cases, would achieve the quantitative conversion of the starting reactant within 3 h (see Scheme 1). Moreover, we developed an operationally simple and versatile workup protocol, which involved recovering high purity final products by filtration upon addition of water to the reaction mixture.

A cell-screening assay was used to test anti-prion activity across the library of synthesized compounds. The ability of **1**–**7a** and **1**–**7b** to reduce PrP^{Sc} concentrations in ScGT1 cells was determined by Western blot densitometry of the PK-resistant PrP^{Sc}. First, we determined the effects of library compounds on cell viability (Table 1). Compound toxicity is expressed as an average percentage of viable cells when treated with a compound concentration of 1 μM, versus control cells treated with no compound. For compound **6a**, the LC_{50} value (lethal concentration, 50%) for ScGT1 cells was also identified. Anti-prion activity is expressed as the average percentage of PK-resistant PrP remaining after incubation with compound at the given concentration, versus control cells incubated with no compound. For entries **6a**, **6b**, and **7a**, we also calculated the EC_{50} values, which represent the effective concentrations for half-maximal inhibition.

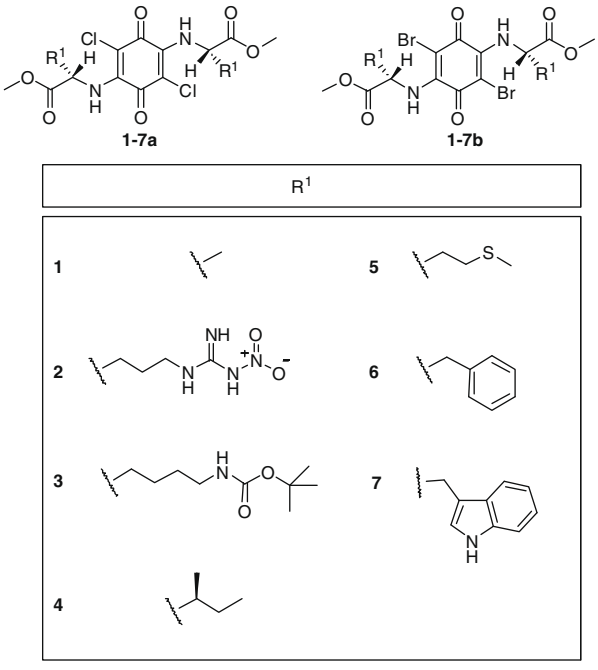
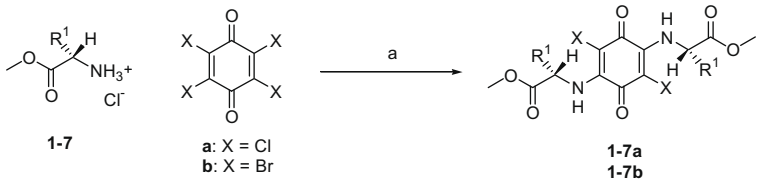


Figure 1. Chemical structures of **1–7a** and **1–7b**.

Analyzing the results in Table 1, we note some interesting trends. The cytotoxic effects of **1–7a** and **1–7b** were first determined by a Calcein-AM assay in the ScGT1 cell line. As reported, treating ScGT1 with the compounds (1 μM) did not significantly modify cell viability. However, treatment with the BOC-Lys derivatives **3a** and **3b** decreased cell viability by percentages of 40% and 52%. Because of the toxicity profile shown **3a** and **3b** were not screened for prion replication, whereas the other library members were assayed at a concentration of 1 μM. Notably, despite this low concentration range, three couples of library hits were active against PrP^{Sc} accumulation. IleOMe (**4a–b),** MetOMe (**5a–b**) and PheOMe (**6a–b**) derivatives of both series at 1 μM displayed activities ranging from 4.8% to 73%. Conversely, AlaOMe (**1a–b**), Nω-Nitro-ArgOMe (**2a–b**), TrpOMe (**7a–b**) derivatives had no effect at that concentration. Due to its low cytotoxicity, **7a** could also be tested at higher concentrations, revealing a fair EC_{50} value of 7.7 μM. For **6a**, we found a remarkable submicromolar EC_{50} value (0.87 ± 0.1 μM; see Fig. 2), comparable to that of quinacrine (0.4 ± 0.1 μM), a reference anti-prion compound.[18,19] The high activity of **6a** and **6b** was not unexpected, as it is in line with the well-known central role of pi-stacking interactions in self-assembly processes in the fields of chemistry and biochemistry.[20]

To better rationalize the obtained results, we applied a systematic procedure for identifying key fragments responsible for a given activity.[21] We used an algorithm which breaks down a structure into fragments.[22] Subsequently, all the obtained substructures were related to biological activities to identify hot fragments. The analysis provides a score [100–0] for each fragment, which gives an indication of how often a fragment occurs in the active and inactive structures (Fig. 3). From this preliminary computational study, we have identified that the 2,5-bisdiaminobenzoquinone linked with two phenyl rings by a spacer is a good anti-prion motif. In addition, our analysis suggests the relevance of the atomic size of the substituents in position 3 and 6 at benzoquinone ring (Cl being better than Br), with an inverse relationship to van der Waals radius. Both items of information can be exploited to design further series of anti-prion small molecules.

Despite the small number of compounds synthesized, the results suggest that some are active against prion replication. Although the existing derivatives were overall quite cytotoxic toward ScGT1 cells, we identified entries **6a** and **6b** as hit compounds for further lead optimization studies. At a time when it remains



Scheme 1. Reagents and conditions: (a), EtOH, NEt_3, 3 h, 30–45% yield.

Table 1
Cell viability and anti-prion activity on ScGT1 cells of library compounds

Cmpd	% of Viable cells at 1 μM[b]	% PrP^{Sc} inhibition at 1 μM[c]	EC_{50}[c] (μM)	LC_{50}[b] (μM)
1a	71.6 ± 5.4[a]	0.36 ± 0.034[a]		
1b	91.2 ± 6.8	0.29 ± 0.05		
2a	81.4 ± 8.7	0.23 ± 0.01		
2b	74.1 ± 4.3	0.31 ± 0.03		
3a	40.7 ± 5.5	ND		
3b	52.3 ± 5.9	ND		
4a	88.2 ± 6.1	6.6 ± 0.4		
4b	91.3 ± 8.8	28.1 ± 1.5		
5a	95.5 ± 6.2	4.8 ± 0.7		
5b	96.1 ± 7.5	11.4 ± 0.5		
6a	68.4 ± 7.3	73.2 ± 3.3	0.87 ± 0.1	2.4 ± 0.2
6b	80.2 ± 5.8	18.1 ± 0.5	3.6 ± 0.5	
7a	96.0 ± 7.6	0.25 ± 0.04	7.7 ± 1.2	
7b	65.9 ± 3.4	0.43 ± 0.01		

[a] Values are the mean of three experiments, standard deviations are given.
[b] ScGT1 cells were cultured in DMEM with 10% FBS, plated 25000 cells in each well of 96-well plates. The compounds were dissolved in DMSO (100%) and diluted in PBS 1X before adding various concentrations (1 nM–10 μM) and incubated for 5 days at 37 °C, 5% CO_2. The results were developed by Calcein-AM fluorescence dye and read by microplate reader.
[c] The effect of library compounds on inhibition of scrapie prion replication. ScGT1 cells were cultured in DMEM with 10% FBS, split 1:10 into Petri dishes and incubated for 2 days at 37 °C and 5% CO_2. Then, various compound concentrations (0.1 nM–1 μM), being non-toxic for the cells, were added to the plates. After a 5-day incubation, proteins of cells were extracted, quantified, digested with proteinase K (PK), and Western-blotted.

challenging to design chemical entities able to target PPIs, these studies might shed light on the underlying principles governing molecular recognition and the chemical basis for the inhibition of quinone derivatives in prions.

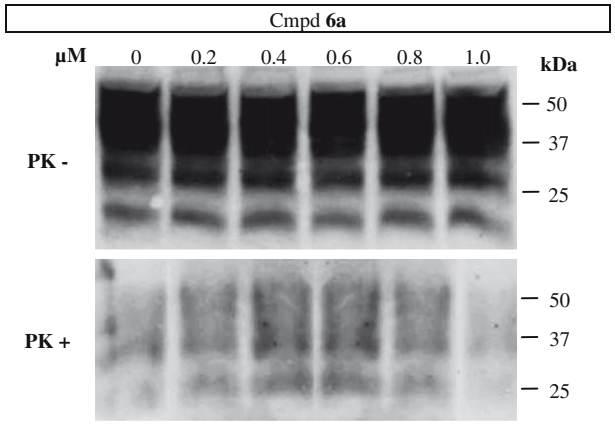


Figure 2. Western blot of protease-digested ScGT1 cell lysates depicting the presence or absence of prions (PrP^{Sc}) after treatment with **6a** before (up) or after (bottom) proteinase K (PK).

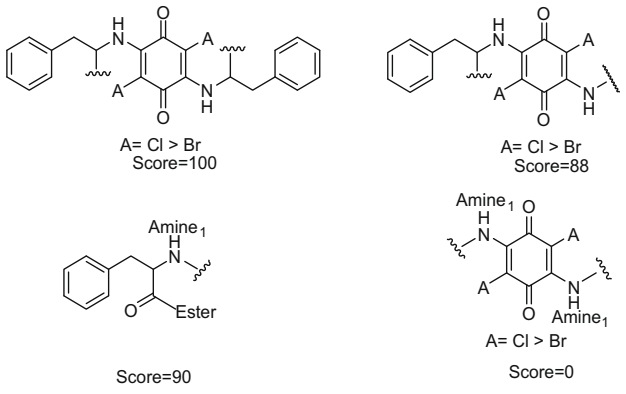


Figure 3. Substructures identified from the synthesized library.

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References and notes

1. Arkin, M. R.; Wells, J. A. *Nat. Rev. Drug Disc.* **2004**, *3*, 301.
2. Forman, M. S.; Trojanowski, J. Q.; Lee, V. M. *Nat. Med.* **2004**, *10*, 1055.
3. Trojanowski, J. Q.; Lee, V. M. *Ann. NY Acad. Sci.* **2000**, *924*, 62.
4. Nooren, I. M.; Thornton, J. M. *Embo J.* **2003**, *22*, 3486.
5. Kranjc, A.; Bongarzone, S.; Rossetti, G.; Biarnes, X.; Cavalli, A.; Bolognesi, M. L.; Roberti, M.; Legname, G.; Carloni, P. *J. Chem. Theory Comput.* **2009**, *5*, 2565.
6. Soto, C. *FEBS Lett.* **2001**, *498*, 204.
7. Soto, C. *Nat. Rev. Neurosci.* **2003**.
8. Gestwicki, J. E.; Marinec, P. S. *Comb. Chem. High Throughput Screening* **2007**, *10*, 667.
9. Haydar, S. N.; Yun, H. E. D.; Staal, R. G. W.; Hirst, W. D. Small-Molecule Protein–Protein Interaction Inhibitors as Therapeutic Agents for Neurodegenerative Diseases: Recent Progress and Future Directions. In *Annual Reports in Medicinal Chemistry*; Elsevier Academic: San Diego, 2009; Vol. 44, p 51.
10. Blazer, L. L.; Neubig, R. R. *Neuropsychopharmacology* **2009**, *34*, 126.
11. Xu, Y.; Shi, J.; Yamamoto, N.; Moss, J. A.; Vogt, P. K.; Janda, K. D. *Bioorg. Med. Chem.* **2006**, *14*, 2660.
12. Caughey, B.; Baron, G. S. *Nature* **2006**, *443*, 803.
13. Soto, C.; Kascsak, R. J.; Saborio, G. P.; Aucouturier, P.; Wisniewski, T.; Prelli, F.; Kascsak, R.; Mendez, E.; Harris, D. A.; Ironside, J.; Tagliavini, F.; Carp, R. I.; Frangione, B. *Lancet* **2000**, *355*, 192.
14. Sellarajah, S.; Boussard, C.; Lekishvili, T.; Brown, D. R.; Gilbert, I. H. *Eur. J. Med. Chem.* **2008**, *43*, 2418.
15. Bartolini, M.; Bertucci, C.; Bolognesi, M. L.; Cavalli, A.; Melchiorre, C.; Andrisano, V. *ChemBioChem* **2007**, *8*, 2152.
16. 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.
17. Pawar, A. P.; Dubay, K. F.; Zurdo, J.; Chiti, F.; Vendruscolo, M.; Dobson, C. M. *J. Mol. Biol.* **2005**, *350*, 379.
18. Yung, L.; Huang, Y.; Lessard, P.; Legname, G.; Lin, E. T.; Baldwin, M.; Prusiner, S. B.; Ryou, C.; Guglielmo, B. J. *BMC Infect. Dis.* **2004**, *4*, 53.
19. Ghaemmaghami, S.; Ahn, M.; Lessard, P.; Giles, K.; Legname, G.; DeArmond, S. J.; Prusiner, S. B. *PLoS Pathog.* **2009**, *5*, e1000673.
20. Gazit, E. *Faseb J.* **2002**, *16*, 77.
21. Lewell, X. Q.; Judd, D. B.; Watson, S. P.; Hann, M. M. *J. Chem. Inf. Comput. Sci.* **1998**, *38*, 511.
22. Fragmenter was used for molecular decomposition to fragments and R-groups, JChem 5.0.0, 2008, ChemAxon (http://www.chemaxon.com).