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Miniperspective

Polyamines in Drug Discovery: From the Universal Template Approach to the Multitarget-Directed Ligand Design Strategy

Carlo Melchiorre,* Maria Laura Bolognesi, Anna Minarini, Michela Rosini, and Vincenzo Tumiatti

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

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Introduction

The search for drugs to save, preserve, and enhance our lives has long posed great challenges to human effort. Since early times, thanks to the pioneering work of alchemists and then medicinal chemists, humanity has benefited enormously from drug discovery. Nowadays, sophisticated procedures for drug synthesis and target elucidation allow us to design molecules with relative ease and with the hope that these new molecules will become effective medicinal drugs.

However, drug designers still face great challenges mainly because there is no agreement on how to do the job correctly. A key issue in drug discovery is the selection of a lead molecule suitable for developing new molecules for treating diseases.[1] Crucially, how many potential drug targets are responsible for the pathogenic factors leading to a given disease?[2] In general, 20th century drug research aimed to discover drugs that could hit a single target thought to be fully responsible for a given disease. This "one-molecule−one-target" paradigm led to the discovery of many successful drugs and will likely lead to many more. However, single-target drugs may be inadequate for diseases with multiple pathogenic factors (for example, neurodegenerative diseases such as Alzheimer's disease (AD[a])). Pharmacological approaches to treating such diseases will need to diverge from the single-target focus of monotherapy.[3−8]

When a single medicine is not sufficient, three pharmaceutical strategies may be followed (Figure 1): (1) multiple-medication therapy (MMT) involving a "cocktail" of two or more drugs; (2) therapy with a multiple-compound medication (MCM), which incorporates two or more drugs into a single formulation; (3) therapy with a single molecule that hits multiple targets relevant for a given disease. Such drugs have been named multitarget-directed ligands (MTDLs). The first two strategies have been widely applied to treat, for example, HIV, cancer, and hypertension. The third is still in its infancy. But MTDL therapy, unlike MMT or MCM, obviates the challenge of administering multiple single-drug entities, which may have different bioavailability, pharmacokinetics, and metabolism. Furthermore, in terms of pharmacokinetic and ADMET optimization, the clinical development of a drug that hits multiple targets should not, in principle, be different from the clinical development of a single-target drug, which is particularly relevant because the pharmacokinetic profiling of a drug candidate is one of the major contributors to the attrition rate in drug development. In addition, MTDL therapy reduces the risk of drug−drug interactions and simplifies the therapeutic regimen with respect to MMT. The above considerations suggest that the development of MTDLs might lead to new pharmaceutical treatments for multifactorial pathologies such as AD, for which effective cures are urgently needed.[9−15]

Lead Selection

Researchers in drug discovery need to know the molecular properties of the target(s) under investigation. They also need to find a lead molecule with an appropriate biophysicochemical profile for absorption, distribution, metabolism, and excretion to prevent resources being squandered on less promising lead molecules. Thus, it is vital to identify lead molecules with recognizable druglike properties to avoid producing active molecules that, a priori, will be theoretically but not pharmaceutically interesting. Selecting a lead molecule for development is difficult and will be different for each target. The task would be much easier if there were a single molecule able to affect any target either positively or negatively. This molecule would thus function as a pharmaceutical skeleton key.

A lead molecule endowed with such properties has been termed a "universal template", as it can provide, through appropriate structural modifications, new molecules with high affinity and selectivity for any target.

Polyamines, a Universal Template?

The concept that a polyamine skeleton may represent a universal template was proposed in 1988,[16,17] following the discovery of the tetraamine disulfide benextramine (**1**) (Figure 3),[18,19] an irreversible α-adrenoreceptor antagonist. Soon after, **1** was shown to recognize other receptor systems, such as nicotinic receptors,[20] muscarinic receptors,[21] and neuropeptide Y receptors.[22,23] Thus, the ability of this particular polyamine to recognize different receptor systems provided the rationale for developing, through appropriate modification of its structure, polyamines selective for different biological targets.[17]

The assumption that a polyamine may serve as a pharmaceutical skeleton key rests on the following: Neurotransmitter receptors share a high percentage of homology, which increases

*To whom correspondence should be addressed. Phone: +39 0512099706. Fax: +39 0512099734. E-mail: carlo.melchiorre@unibo.it.

[a]Abbreviations: Aβ, amyloid-β; ACh, acetylcholine; AChE, acetylcholinesterase; AChEI, acetylcholinesterase inhibitor; AD, Alzheimer's disease; CoQ, coenzyme Q; GPC1, glypican 1; MCM, multiple-compound medication; MMT, multiple-medication therapy; MTDL, multitarget-directed ligand; NMDA, *N*-methyl-D-aspartate; NQO1, NAD(P)H/quinone oxidoreductase 1; ORT, object recognition test.

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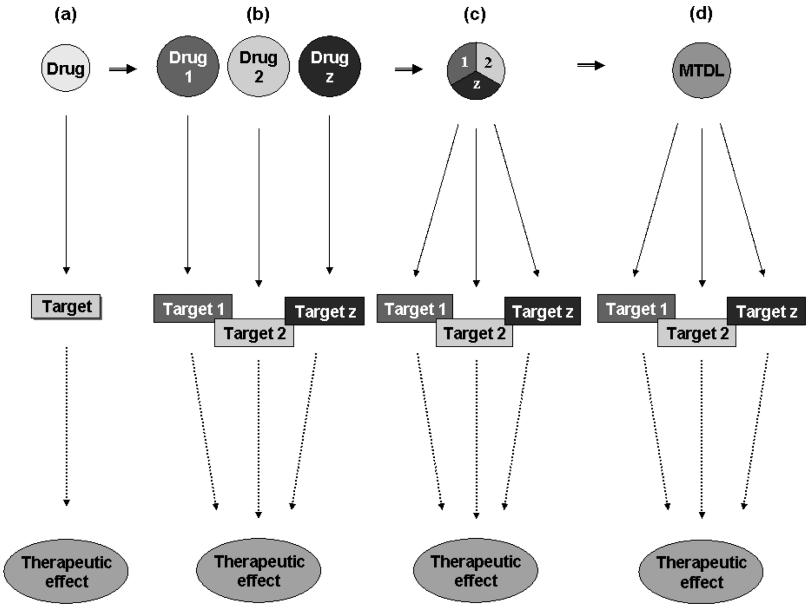

Figure 1. Therapeutic strategies: evolution of monotherapy (a) to therapy with different drugs forming an MMT (b) or an MCM (c) or with a single drug molecule able to modulate multiple targets (d). Strategies depicted in (b), (c), and (d) should, in principle, produce the same therapeutic effect in treating a given disease. However, only (d) avoids the risk of drug−drug interactions that are possible with (b) and (c).

within the subtypes of a given receptor family, accounting for the difficulty of achieving drug selectivity for a specific receptor. However, receptor homology may be exploited to design molecular entities that can, in principle, recognize different receptor systems. This is because neurotransmitter receptors are folded polypeptide chains, which always contain the same amino acids, albeit in different proportions and sequences. The sequence of these amino acids constitutes the primary structure, which is derived from peptide bonds linking carboxylate groups to amino groups. The resulting units (i.e., the peptide bond and the α carbon) are repeated to form a chain, the so-called "backbone" of a protein. We know that the backbone of a protein has mainly a structural role (that is, it cannot be considered a target for drug selectivity). It therefore follows that it is the chains lateral to the backbone that play the major role in binding drugs to receptors. Of these chains, aspartate, glutamate, and aromatic residues are particularly important for binding with cationic ligands by way of a cation−anion or a cation−π interaction. A protein may bear several carboxylate and/or aromatic residues somewhere in its structure. Therefore, in principle, it should be possible to design a lead molecule with a polyamine backbone that is able to recognize multiple anionic sites of a given receptor. Such a molecule would be able to interact with all receptor proteins, provided that the distance separating the amine functions of the molecule fitted the distance between the carboxylate or aromatic residues of the receptor. In other words, a polyamine could be considered a skeleton key in the drug−receptor recognition process because it can assume different conformations in order to enable interaction between protonated amine functions and receptor anionic sites.

Why a Polyamine?

As mentioned above, the selection of a suitable lead molecule is crucial in drug discovery. The final goal of this process should be a drug candidate for clinical trials with the hope of affording an effective medicine for a given disease. Therefore, a lead molecule should possess druglike properties to increase

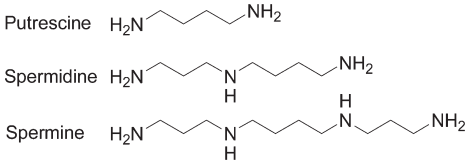

Figure 2. Some naturally occurring polyamines.

the chances of producing, through appropriate structure−activity relationship studies, drug candidates. In this context, the huge variety of naturally occurring polyamines indicates that they are "privileged structures"[24,25] validated by nature. The most common natural polyamines, such as putrescine, spermidine, and spermine (Figure 2), are aliphatic molecules with amine groups distributed along their structure. These polyamines are present in all organism cells, where they play a fundamental role in cell proliferation and have both pro- and antiapoptotic effects.[26,27] Additionally, polyamines are involved in many signaling pathways through their effects on G proteins, protein kinases, nucleotide cyclases, and receptors and through their regulation of the expression of proteins involved in these processes.[26−32] Because of their interactions with certain transmembrane ion channels, they also influence the electrical properties of excitable cells.[33] Spermine is also released from synaptic vesicles on depolarization, indicating that polyamines may function as neuromodulators.[34] Moreover, polyamines influence the properties of several neurotransmitter pathways known to be involved in mental disorders, including catecholamines, γ-aminobutyric acid, nitric oxide, and glutamate.[26]

The wide range of activities displayed by polyamines is clear evidence of how nature exploits them for different roles at different targets, supporting the viability of polyamine skeletons as lead molecules.

Polyamines may be endogenous or exogenous. Since exogenous polyamines are dietary,[30] it is evident that they are efficiently absorbed following oral assumption. Multiple transport systems have been identified in various cell types,

suggesting that polyamines can be widely distributed throughout the body to fulfill their biological roles.[31,35,36] One model sees polyamines transported into cells through unidentified membrane transporters/carriers driven by a membrane potential and sequestered into vesicles by proton exchange over a pH gradient built by a vacuolar ATPase.[37,38] Another model proposes a role for the heparin sulfate side chains of recycling glypican 1 (GPC1) in the transport of spermine and assumes that GPC1 recycling is the basis of polyamine transport.[39] However, other mechanisms have also been proposed for polyamine transport.[40]

As well as naturally occurring polyamines, there are many synthetic polyamines with a linear backbone. These ligands affect different biological targets, which may be either activated or inhibited, suggesting that polyamine research is an important field for drug development with great potential for identifying new molecules for different diseases. There are several review articles on polyamines as antiproliferative agents[41] and as neurotransmitter receptor agonists/antagonists,[42,43] neuroprotectants,[44] anti/prion chemotherapeutics,[45] and potent antiparasitic compounds.[46,47]

Modulating the Affinity and Selectivity of a Polyamine Skeleton

It has been reported that polyamines, such as spermine and homospermine, are highly protonated at physiological pH. Spermine and homospermine are 85% and 97% tetracation, respectively, the remainder being the trication.[48] Thus, the ability of a polyamine to interact with biological counterions (these are, for example, the one or more sets of carboxylate anions fixed to the backbone of a protein) could be related to its cationic properties. Consequently, the distance between the cationic nitrogen atoms of a polyamine is critical for drug recognition.

We know that increasing the number of interactions between a receptor (protein) and a ligand increases the chances of a ligand being able to distinguish between different receptor systems. Thus, an appropriate modification of the chain length separating the nitrogen atoms of a polyamine might increase affinity, while the insertion of N-substituents might improve both affinity and selectivity by increasing the overall number of contacts between a drug and a receptor.

Universal Template Approach vs Drug Design

The application of the universal template approach can be exemplified by the development of polyamines as selective antagonists for muscarinic receptor subtypes and nicotinic receptors using **1** as the focus. **1** covalently inhibited α-adrenoreceptors through bond formation between a receptor thiol and the disulfide bridge of the inhibitor via a disulfide−thiol interchange reaction.[14] **1** also competitively antagonized muscarinic receptor subtypes, albeit with modest affinity. The finding that muscarinic receptor inhibition was not dependent on the disulfide moiety prompted the development of polyamines that were selective for muscarinic receptor subtypes while losing affinity for α-adrenoreceptors. Briefly, as well as the replacement of the disulfide bridge by two methylenes, three types of structural modifications were performed on **1**: (1) variation of the carbon chain length separating inner from outer nitrogen atoms (distance *m*) and inner nitrogen atoms (distance *n*), (2) incorporation of substituents on the four nitrogen atoms, and (3) variation in the number of nitrogen atoms.

The design strategy is illustrated in Figure 3. Investigation of the chain length showed that both distances *m* and *n* are important for activity. This study led to the discovery of methoctramine (**2**),[49] which is the prototype of polymethylene tetraamines as muscarinic receptor antagonists. It is able to significantly distinguish between atrial muscarinc M_2 receptors and ileal muscarinic M_3 receptors. Further SAR studies revealed that an appropriate decoration of the four nitrogen atoms of polymethylene tetraamines may significantly affect the biological profile. For example, the insertion on the terminal nitrogen atoms of **2** of the tricyclic moiety (11-acetyl-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepine-6-one) of pirenzepine afforded, among other tetraamines, the nonsymmetrically substituted tetraamine tripitramine (**3**),[50] which was one of the most potent and selective muscarinic M_2 receptor competitive antagonists thus far available. In contrast, replacing the terminal amine functions of **2** with a 3,3-diphenyl-1,4-dioxa-8-aza-spiro[4.5]decan-2-one moiety led to the symmetrically substituted spirotramine (**4**),[51] which displayed an inverse selectivity profile in comparison with both **2** and **3**. This is because of a higher affinity for muscarinic M_1 receptors and a significantly lower affinity for all the muscarinic receptor subtypes investigated. As the universal template approach would suggest, this finding clearly supported the view that both affinity and selectivity can be tuned by inserting appropriate substituents onto the amine functions of a polyamine backbone (Figure 4).

Concerning the role of the number of basic nitrogen atoms of **2** and **3**, it emerged that they were necessary for both affinity and selectivity. Splitting the structure of **3** in two afforded diamines that were 3−4 orders of magnitude less potent than the progenitor at guinea pig left atria muscarinic M_2 receptors, with complete loss of selectivity over the ileal M_3 subtype.[50] This paralleled the results observed with **2**. Removing one or two nitrogen atoms from the tetraamine backbone caused a dramatic drop in affinity for muscarinic M_2 receptors, confirming that the number of basic nitrogen atoms is a primary requisite for discriminating among muscarinic M_2 receptors and the other muscarinic receptor subtypes. Since the four nitrogen atoms of **2** are fully protonated at physiological pH, it is derived that they could interact with four anionic sites. The interaction of **2** with muscarinic receptor subtypes was rationalized as follows: two nitrogen atoms would bind at two of the three Asp residues (namely, Asp 97, 103, and 120, following the porcine cardiac sequence numbering) which are conserved in the third transmembrane domain of all muscarinic receptor subtypes. The other two nitrogen atoms of **2** (as well as those of related tetraamines) would interact with the two anionic sites not conserved in muscarinic receptor subtypes, which might explain the selectivity, as an example, of **3** for a specific subtype (Figure 5).[52]

The universal template approach has been applied not only to G-protein-coupled receptors, as outlined above, but also to the muscle-type nicotinic receptor, a prototypical member of the superfamily of ligand-gated ion channels. The tetraamine backbone of **2** has been extensively modified affording symmetrically and unsymmetrically substituted polyamines displaying affinity for muscle-type nicotinic receptors. Of these polyamines, photoaffinity labels MR44 (**5**) and DMR44 (**6**) and their corresponding iodine derivatives ^{125}I-**5** and $^{125}I_2$-**6** (Figure 3) proved to be a most promising tool for studying the binding of a polyamine to nicotinic receptors. Researchers found that the unsymmetrically substituted ^{125}I-**5** interacted with the receptor with a 2:1 stochiometry, while the

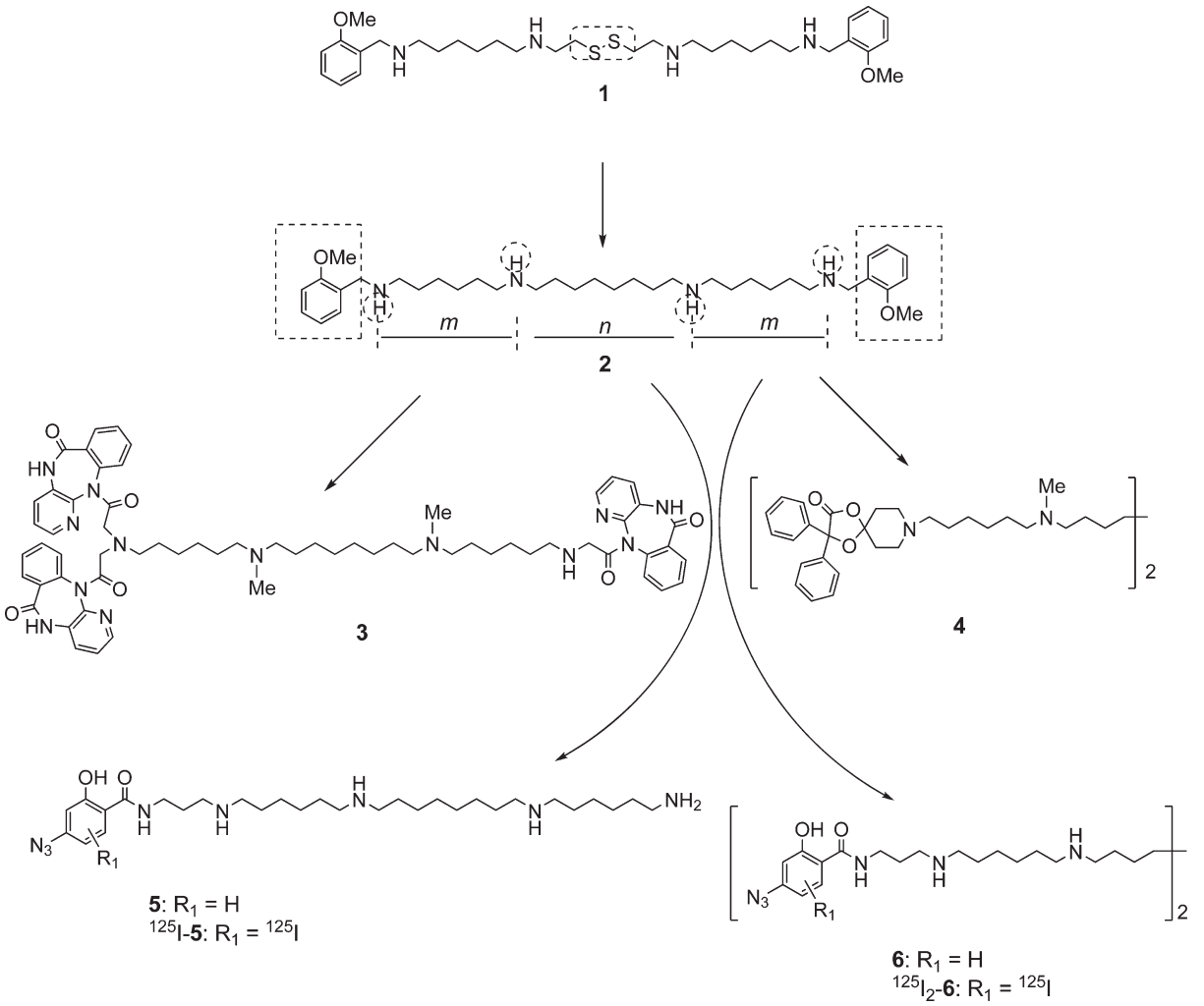

Figure 3. Structural modifications on benextramine (**1**) structure leading to polymethylene tetraamines selective for different receptor systems. *m* and *n* represent the carbon chain length separating inner from outer nitrogen atoms and inner nitrogen atoms, respectively. **1** is a covalent nonselective inhibitor of α-adrenoreceptors, whereas methoctramine (**2**), tripitramine (**3**), and spirotramine (**4**) are selective and competitive antagonists of muscarinic receptors with an affinity profile $M_2 \geq M_4 \geq M_1 \gg M_3$, $M_2 \gg M_4 = M_1 \gg M_3$, and $M_1 \gg M_4 = M_2 = M_3$, respectively. MR44 (**5**), DMR44 (**6**), and their iodine derivatives are noncompetitive antagonists of the closed channel conformation of the muscle-type nicotinic receptor.

symmetrically substituted $^{125}I_2$-**6** interacted with a 1:1 stochiometry. It was thus suggested that, at least with Torpedo nicotinic receptors, symmetrically substituted tetraamines interact differently from monosubstituted tetraamines that bear a terminal primary amine function (Figure 6).[53]

Interestingly, it was found that the interaction between target receptors and **2** and its derivatives is evident at nanomolar concentration, whereas cytotoxicity is detectable in the micromolar range, making their pharmacological use in biological models safe enough.[54]

Polyamine Backbone and the MTDL Design Strategy

The ability of the polyamine backbone to hit any target is a step forward for the universal template concept. In principle, a universal template should recognize, albeit with low affinity, many different targets. Appropriate structural modifications should produce molecules with affinity for a single given target. Moving from the target-centric strategy to the MTDL approach should produce molecules with affinity for selected multiple targets while having low or no affinity for the other targets. Thus, a lead molecule may be a universal template in either the "one-molecule−one-target" or the "one-molecule−multiple-targets" strategies. The assumption that a polyamine skeleton may be a suitable MTDL lead rests on the following: Through interaction with anionic or aromatic sites, the polycationic nature of the polyamine skeleton will ensure recognition of macromolecules such as DNA, RNA, and proteins with unpredictable affinity and selectivity over nontargets. The crucial step is then achieving selectivity for targets relevant to a specific multifactorial disease while reducing or, hopefully, abolishing affinity for nontargets. With MTDL design strategies still in their infancy, the paucity of MTDL structure−activity relationship studies makes this a difficult goal. However, we can use the available information on the structural requirements for hitting the single targets involved in a given multifactorial disease. It should thus be feasible to design potential MTDLs by inserting appropriate pharmacophores (relevant for each of the selected targets) on the nitrogen atoms or on the spacer connecting these atoms on a polyamine skeleton. This structural modification should result in an overall increase of the number of contacts with selected targets, improving both affinity and selectivity. In other words, if a polyamine can be modified to achieve

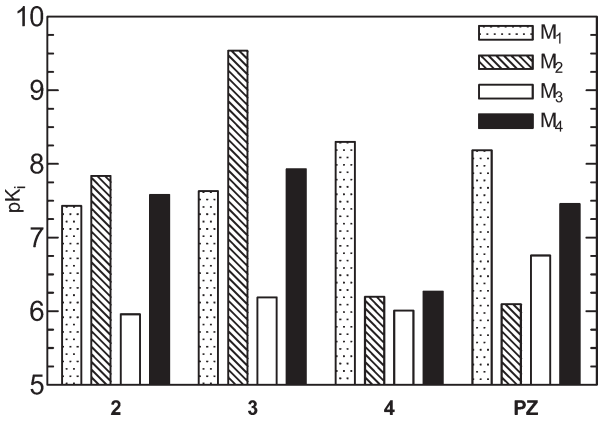

Figure 4. Muscarinic receptor subtype selectivity profile of polymethylene tetraamines methoctramine (**2**), tripitramine (**3**), and spirotramine (**4**) in comparison to pirenzepine (PZ). Affinity constants (pK_i) were obtained in rat cortex (M_1), heart (M_2), and submaxillary gland (M_3) and NG 108-15 cell (M_4) muscarinic receptors (data from refs 50 and 51).

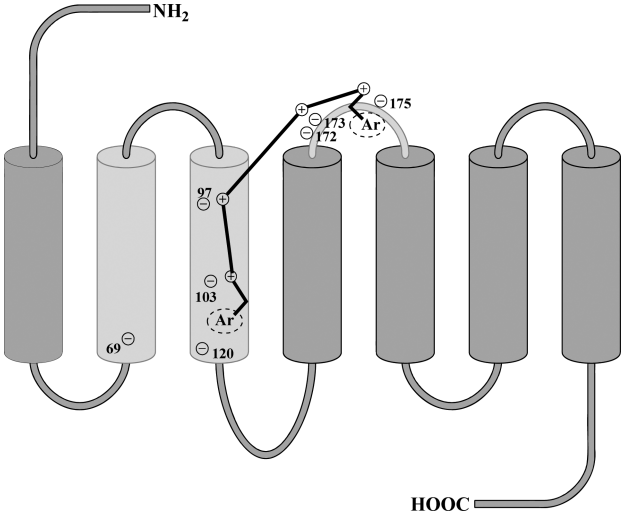

Figure 5. Schematic representation of the interaction between polymethylene tetraamines and muscarinic M_2 receptors. The acidic residues, aspartates 69, 97, 103, and 120 in transmembrane domains II and III, and aspartate 173 and glutamates 172 and 175 in the extracellular loop 4−5, are indicated by ⊖ to denote their anionic nature. **2** is represented with a bold line connecting four positive charges (the four basic nitrogen atoms) and ending with Ar (2-methoxybenzyl groups). The primary event of the interaction might take place between a terminal nitrogen atom of **2** and glutamate 175 and aspartate 173 (or glutamate 172) on the extracellular loop 4−5. This binding would be reinforced by the interaction of Ar with an appropriate area (broken circle) and would cause, by way of a conformational change, the penetration of **2** into the third transmembrane domain of the receptor followed by interaction with the aspartates 97 and 103. This hypothetical mode of action would not apply to other muscarinic receptor subtypes because aspartate 173 and glutamates 172 and 175 are not conserved, which may explain the selective binding of **2**. The figure is adapted from Melchiorre et al.[52]

selectivity for a specific target, it follows that, in principle, the same lead molecule could be appropriately modified to afford chemical entities that simultaneously modulate multiple targets relevant for a given multifactorial disease such as AD. Nowadays, it is clear that AD, a devastating form of dementia, is a multifactorial pathology caused by genetic, environmental, and endogenous factors, including excessive protein

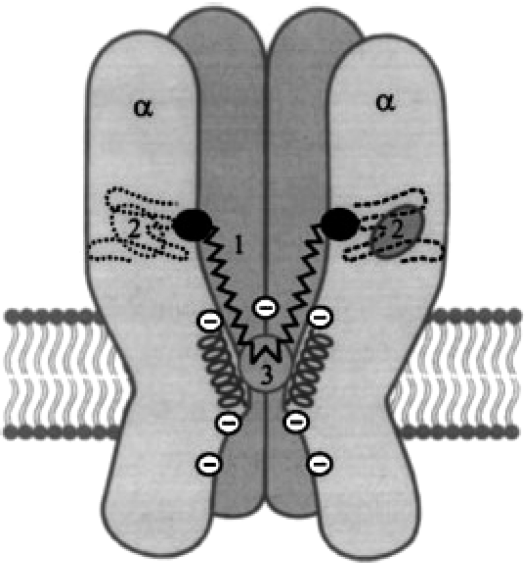

Figure 6. Binding mode of symmetrically substituted tetraamines at the Torpedo muscle-type nicotinic receptors: (1) $^{125}I_2$-**6**; (2) agonist binding site; (3) high affinity noncompetitive binding site. Each of the two aromatic moieties of $^{125}I_2$-**6** most likely interacts with one of the two α-subunits of the nAChR monomer. The long positively charged polyamine chain, which overlaps the noncompetitive inhibitor site, reaches further down into the ion channel and is located close to the negatively charged selectivity filter. Reprinted from *Journal of Medicinal Chemistry*.[53]

misfolding and aggregation, oxidative stress and free radical formation, impaired bioenergetics and mitochondrial abnormalities, and neuroinflammatory processes. Despite its huge effects, AD remains incurable and fatal.[55,56] The commercially available therapeutic options are the four acetylcholinesterase (AChE) inhibitors (AChEIs) donepezil, rivastigmine, galantamine, and tacrine (retired, however, from the market) and the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine. None can alter or prevent disease progression. This is because they are monofunctional, designed to hit only a single target among the many involved in AD's pathogenesis (Figure 7). Therefore, AD remains an area of exceptional clinical need.

The development of memoquin (**8**),[57−59] a drug candidate for AD treatment, illustrates the MTDL design strategy (Figure 8). An MTDL hitting more than one specific target offers a better pharmacological approach to AD. The design of polyamines as anti-AD molecules was based on **1**, which was assumed to be a suitable lead molecule because of its multiple biological properties, such as the ability to hit AChE and muscarinic M_2 receptor, two targets relevant to AD pathogenesis. The inhibition of AChE would increase the concentration of acetylcholine (ACh), whereas the antagonism of the presynaptic muscarinic M_2 receptor would favor the release of ACh in the synapse leading to an improvement of the cholinergic transmission and, consequently, an improvement of the cognition that is compromised in AD pathology. An appropriate structural modification of **1** afforded caproctamine (**7**), which was endowed with a balanced biological profile toward AChE (pIC_{50} = 6.77) and muscarinic M_2 receptors (pA_2 = 6.39).[60] **7** was also shown to bind both the catalytic and peripheral sites of AChE. This was relevant because the peripheral site of AChE had been shown to be involved in the interaction with β-amyloid (Aβ) with subsequent amyloid fibril aggregation, another event in the AD

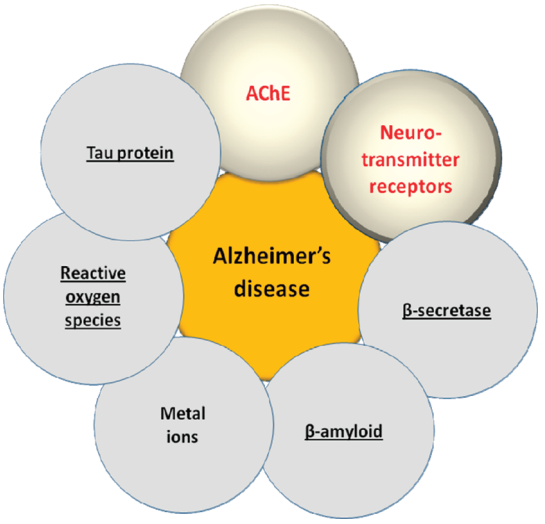

Figure 7. Schematic illustration of some of the multiple pathways that have been recognized as fundamental in AD pathogenesis. The currently available drugs, namely, AChEIs (tacrine, galanthamine, donepezil, and rivastigmine) and noncompetitive NMDA receptor antagonists (memantine), hit the targets in red, while the MTDL **8** modulates both AChE and the underlined targets/pathways.

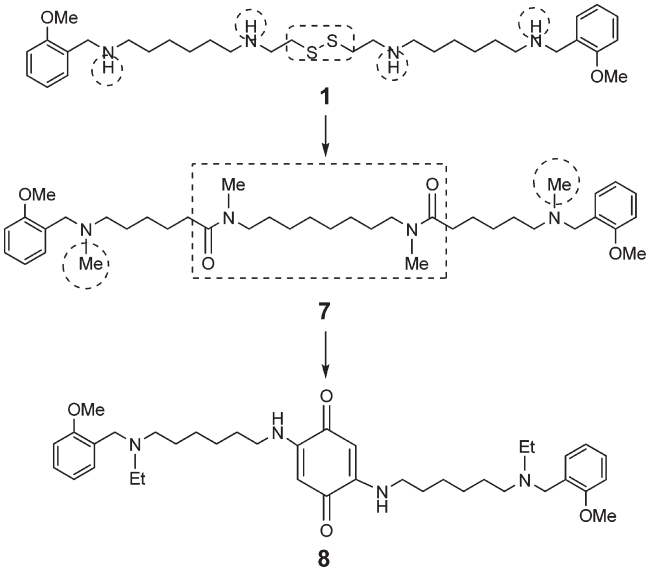

Figure 8. Design strategy leading to **8**.

pathogenesis cascade.[61−63] Thus, **7** became the new lead molecule for designing better anti-AD polyamines that would hopefully display additional biological activities. To this end, the highly flexible octamethylene spacer of **7** was replaced with the 1,4-benzoquinone radical scavenger moiety of coenzyme Q (CoQ) and idebenone, a synthetic analogue of CoQ. This choice was dictated by the observation that CoQ improved cognitive function and behavioral deficits in patients with mild to moderate AD and protected the hippocampus neurons against Aβ-induced neurotoxicity.[64,65] Thus, appropriate modifications performed on the **7** structure afforded **8**, which has been investigated both in vitro and in vivo to assess its potential as a drug candidate for AD treatment.[57,58]

The in vitro activities of **8** are presented in Table 1. As expected, **8**, like caproctamine, inhibited AChE with a potency 10-fold higher than that of donepezil, the most potent marketed AChEI. Since **8** was shown to simultaneously bind

Table 1. In Vitro Activities of **8** at the Selected Molecular Targets in Comparison with a Reference Compound[a]

target	biological activity
NQO1	**8**: V_{max} = 3480 (μM/min)/mg; K_M = 12.7 μM
	menadione: V_{max} = 7290 (μM/min)/mg; K_M = 1.20 μM
AChE	**8**: IC_{50} = 1.55 ± 0.11 nM; K_i = 2.60 ± 0.48 nM
	donepezil: IC_{50} = 23.1 ± 4.8 nM; K_i = 20.5 ± 3.3 nM
AChE-induced Aβ aggregation	**8**: IC_{50} = 28.3 ± 0.30 μM
	donepezil: $IC_{50} \gg 100$ μM
Aβ self-aggregation	**8**: IC_{50} = 5.93 ± 0.33 μM
	tetracycline: IC_{50} = 60.3 ± 11.2 μM
β-secretase	**8**: IC_{50} = 108 ± 23 nM
	statine derivative: IC_{50} = 18 ± 2 nM

[a] Data from refs 57 and 58.

both the catalytic and peripheral sites of AChE, its ability to inhibit AChE-induced Aβ aggregation was investigated. It turned out that **8** prevents Aβ aggregation to a significantly higher degree than donepezil. Since the 1,4-benzoquinone moiety might be responsible for inhibiting the self-promoted Aβ aggregation,[66] **8** was studied in self-aggregation experiments of Aβ(1−42), the most amyloidogenic fragment found in the AD plaques. Interestingly, **8** exhibited a strong dose-dependent inhibitory effect and, in the same experimental conditions, other AChEIs, such as galantamine and tacrine, did not show significant inhibitory activity.[58] As outlined in the design strategy, the 1,4-benzoquinone moiety was introduced into the **8** structure with the aim of retaining the antioxidant properties of CoQ.

In this regard, we note that the antioxidant properties of 1,4-benzoquinone-bearing compounds, such as CoQ, are due to their hydroquinone forms, since the quinone, in principle, cannot scavenge radicals. The enzyme NAD(P)H/quinone oxidoreductase 1 (NQO1) has been shown to be responsible for the regeneration and maintenance of the CoQ-reduced state, providing a shunt that competes with the formation of free radicals. NQO1 is overexpressed in AD in response to the shift of redox balance typical of the pathology.[67,68] Interestingly, **8** emerged as a good substrate of NQO1, being similar to menadione in terms of reduction by the enzyme. The antioxidant properties of **8** were confirmed in SH-SY5Y neuroblastoma cells pretreated with sulforaphane, a potent inducer of NQO1, and following treatment with *tert*-butyl hydroperoxide. **8** produced a remarkable inhibitory effect on the reactive oxygen species formation relative to the untreated (sulforaphane) cells, confirming the direct relationship between the NQO1-mediated reduced form of **8** and its ability to prevent free radical formation and damage. Finally, **8** was found, unexpectedly, to inhibit the enzyme β-secretase, which together with γ-secretase is responsible for the formation of Aβ through the cleavage from the amyloid precursor protein. Clearly, this finding is very promising for a potential drug candidate.[69]

The in vivo biological profile of **8** was assessed in the AD11 mouse, which has been proposed as a comprehensive animal model for AD.[70] The profile can be summarized as follows:[57−59,71] in AD11 mice aged 15 months, an age at which the disease is fully developed, **8** prevented cholinergic deficit in the basal forebrain and decreased the number of Aβ plaques in comparison with placebo-treated mice. Furthermore, **8** completely (at 2 months of age) or partially (at 15 months of age) prevented the accumulation, in the somadendritic

compartments of AD11 mice, of intracellular tangles composed of the hyperphosphorylated form of the tau protein, another major neuropathological hallmark of AD. The efficacy of **8** in rescuing behavioral deficits linked to attention and memory was also confirmed through the object recognition test (ORT) by pretreating the mice with **8** prior to administrating scopolamine. Finally, the multiple biological properties of **8** have been compared with those of a benchmark AChEI (galantamine) and the lead molecule caproctamine in rescuing the AD phenotype in AD11 mice. Galantamine rescued the cholinergic deficit, the accumulation of Aβ in dystrophic neuritis, and the behavioral ORT deficit but had no effect on the tau phenotype. Unexpectedly, caproctamine had weak or no effect on the number of cholinergic neurons and on the Aβ deposition and tau hyperphosphorylation. Only **8** was able to affect the whole range of hallmarks that characterize AD-like neurodegeneration in AD11 mice.[59] Thus, a rationally designed MTDL has a better chance of affecting overall AD neurodegeneration by acting on multiple targets at different levels of the neurotoxic cascade. Although we have only preliminary, unpublished results on the pharmacokinetics of **8**, its in vivo activity in an animal model allows us to assume quite safely that it is able to cross the blood−brain barrier, reaching an effective concentration in the CNS.

In conclusion, the biological profile of **8** supports the view that a polyamine backbone may serve as a master key for developing new chemical entities (MTDLs) able to simultaneously hit multiple biological targets.

Conclusions

Drug discovery has greatly improved humanity's quality of life. It has been based mainly on the "one-molecule−one-target" paradigm. But drugs modulating a single target are unlikely to be effective in treating multifactorial diseases. The MTDL approach offers a promising new strategy for discovering small molecules able to address the biological complexity of diseases, including neurodegenerative pathologies.

But whatever the design strategy, the choice of a suitable lead molecule is crucial. A polyamine backbone may represent a universal lead molecule for both the "one-molecule−one-target" and the '"multitarget-directed-ligand" approaches for three main reasons: (a) the abundance of naturally occurring polyamines suggests these templates are validated by nature and may thus be considered "privileged" structures; (b) multiple transport systems have been identified that allow polyamines to be distributed throughout body; (c) their cationic nature at physiological pH enables interaction with any target bearing anionic sites, such as proteins and nucleic acids. However, the rules for developing an effective MTDL are not so clear. Multifactorial diseases are the result of several steps, and addressing the molecular dynamics of disease progression with a single molecule is not an easy task. This is because we should ideally assess the relationship between the progression timeline and a specific molecular target. However, despite the formidable challenge of developing effective multimodal medicines, the MTDL design strategy will likely represent a new paradigm for drug discovery in the future.

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Biographies

Carlo Melchiorre received his degree in Chemistry from Camerino University (1966) where was appointed Full Professor (1980). In 1988, he joined the Faculty of Pharmacy at the University of Bologna where he is presently Professor of Medicinal Chemistry. He has longstanding interests in neurotransmitter receptors. From 1975 to 1979, he joined Professor Bernard Belleau's research group at McGill University (Montreal, Canada) where he developed a series of irreversible α-adrenoceptor antagonists, whose prototype, benextramine, has become a valuable tool in α-adrenergic pharmacology. In 1987, his group designed one of the first selective muscarinic M_2 receptor antagonists, methoctramine, which has been widely used in the characterization of muscarinic receptor subtypes. He is currently focusing his research efforts on the identification of MTDLs for the treatment of neurodegenerative disorders.

Maria Laura Bolognesi graduated from the University of Bologna with a degree in Medicinal Chemistry in 1990 and then moved to the Sigma Tau Industries, Rome, as Research Scientist at the Department of Chemical Research. In 1996, she received her Ph.D. in Pharmaceutical Sciences from the University of Bologna, Italy, under the direction of Carlo Melchiorre. After postdoctoral studies at University of Minnesota in Philip S. Portogheses's laboratory, she returned to the University of Bologna where she was appointed Assistant Professor in 1998 and then Associate Professor of Medicinal Chemistry in 2005. In 2009, she was invited as Distinguished Visiting Professor at the Universidad Complutense of Madrid, Spain, working with Jose Carlos Menendez.

Anna Minarini graduated in Medicinal Chemistry from the University of Bologna, Italy, in 1987 and received her Ph.D. in Pharmaceutical Sciences in 1992 from the same university. In 1990−1991, she was a Visiting Scientist at the University of Buffalo, NewYork. In 1998, she was appointed Associate Professor of Medicinal Chemistry at the Department of Pharmaceutical Sciences of the University of Bologna. Her current research interests are devoted to the design and synthesis of new chemical entities against neurodegenerative diseases.

Michela Rosini obtained her degree in Medicinal Chemistry in 1997, followed by a Ph.D. in Pharmaceutical Sciences in 2001 from the University of Bologna, Italy. In 1998 and 2000, she spent some months at The Royal Danish School of Pharmacy of Copenhagen, Denmark. At present, she is a researcher and Assistant Professor in the Department of Pharmaceutical Sciences of the University of Bologna, focusing her research on the design and synthesis of multitarget-directed ligands for the treatment of Alzheimer's disease.

Vincenzo Tumiatti obtained his degree in Medicinal Chemistry in 1986 from the University of Bologna, Italy. In 1991−1992, he was a Visiting Scientist at the University of Frankfurt/M, Germany. In 2005, he was appointed Full Professor of Medicinal Chemistry in the Department of Pharmaceutical Sciences of the University of Bologna. His current scientific activity is focused on the design and synthesis of new chemical entities against neurodegenerative diseases and cancer.

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