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From kinase inhibitors to multitarget ligands as powerful drug leads for Alzheimer’s disease using protein-templated synthesis

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Abstract: Multitarget directed ligands (MTDLs) are arising as promising tools to tackle complex diseases. The main goal of this work is to create powerful modulating agents for neurodegenerative disorders. To achieve this aim, we have combined fragments that inhibit key protein kinases involved in the main pathomolecular pathways of Alzheimer’s disease (AD) such as tau aggregation, neuroinflammation and decreased neurogenesis, whilst looking for a third action in beta-secretase (BACE1), responsible of β-amyloid production. We obtained well-balanced MTDLs with in vitro activity in three different relevant targets and efficacy in two cellular models of AD. Furthermore, computational studies confirmed how these compounds accommodate adequately into the long and rather narrow BACE1 catalytic site. Finally, we employed in situ click chemistry using BACE1 as protein template as a versatile synthetic tool that allowed us to obtain further MTDLs.

Introduction

The lack of effective treatments for severe diseases characterized by a multifactorial etiology or therapeutic resistances, such as neurodegenerative disorders, cancer or bacterial infections, has led to the emergence of a new drug design paradigm: the multitarget approach strategy. Multitarget directed ligands (MTDLs) are characterized for showing activity in more than one molecular target taking advantage of multiple additive or synergic pharmacodynamic activities in a single molecule. This novel approach, that carefully selects polypharmacological drugs, contrasts with the previous classical design where drugs were designed to modulate a specific target with high selectivity, considering the multiple modulations of several targets undesirable. The single-target molecule strategy has failed in the treatment of several pathologies including infectious diseases, cancer and neurodegenerative disorders and seems no longer appropriate. In order to address these pathologies, polypharmacy has been used in the clinical settings for numerous years targeting several biological systems at the same time. For example, it has been used in the treatment of cardiovascular diseases, cancer or HIV. However, the combination of different drugs for pharmacological treatment entails several risks and raises many difficulties such as unpredicted drug-drug interactions or multiplied side effects in addition to lower patient compliance. With the specific aims of tackling several pathomolecular pathways simultaneously while reducing risks associated to polypharmacy, MTDLs are arising as an ideal strategy for the treatment of complex diseases.

Neurodegenerative diseases, and specifically Alzheimer’s disease (AD), would greatly benefit from a MTDL approach. These complex diseases are characterized by their unknown etiology, their intricate molecular pathology and their multifactorial nature. AD is highly prevalent, and despite the great efforts to find an effective drug, it still presents high clinical failure. Furthermore, the complexity of the molecular pathology suggests that traditional drugs will not be able to produce a therapeutic effect. Due to this evidence, the association of multitarget compounds and neurodegenerative diseases has been growing in recent years and therefore some multitarget compounds have reached clinical trials. The specific design of these compounds needs to be carefully studied, being the main optimal characteristics the following: a) having a similar potency in all the modulated targets in order to enable a proper dose to tackle every pathobiological pathway efficiently, b) targeting proteins that present synergistic

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or additive effects reached with moderate activity that provides the opportunity to encounter a better safety profile, and c) preserving drug-like properties.

Specifically, the main pathological processes in AD are neurodegeneration, protein aggregation and brain inflammation. The major pathological aggregate hallmarks are β-amyloid plaques and neurofibrillary tangles. β-Amyloid plaques are extracellular neurotoxic deposits of β-amyloid peptide caused, among other factors, by an abnormal processing by the beta-secretase (BACE1). Neurofibrillary tangles are intracellular deposits composed of hyperphosphorylated tau protein. Multiple kinases including GSK3β, LRRK2 and CK1ε, among others, are involved in this pathological aggregation.11,12 Currently, different BACE1 and protein kinase inhibitors have reached clinical trials but none has been approved by the regulatory agencies so far.13,14 Our goal is to design multitarget compounds able to interfere simultaneously with three different targets involved in AD: BACE1 and, at least, two different protein kinases. In that sense, the new compounds could interfere with both proteinopathies present in AD. Moreover, since kinase inhibitors are also involved in the reduction of neuroinflammation and beta-amyloid is emerging as a risk factor for many other diseases associated with aging,15,16 the combination of all the biological activities in a single molecule could create powerful drugs not only for AD but also for other dementias associated to frailty and aging. Several bifunctional molecules have already been discovered for the potential treatment of AD, however molecules able to combine 3 biological functions are more scarce. The first reported trifunctional molecules exhibited acetylcholinesterase (AChE) inhibitory activity together with the reduction of AChE-induced amyloid-β aggregation and metal chelating properties.17 Other discoveries have found molecules that tackle relevant AD targets such as AChE and its induced β-amyloid aggregation, butyrylcholinesterase, monoamine oxidase or metal chelation.18,19,20

The rational design of MTDs is based in three main strategies: linkage, fusion and incorporation (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Different strategies used to generate multitarget compounds.

Although the increase in molecular size and weight usually present in the design of multitarget compounds is one of the major disadvantages,21 the long and rather narrow size of the BACE1 catalytic site appears to be suitable for the linkage strategy in a MTD design. The volume gained by linking two different protein kinase inhibitors could adjust into BACE1 catalytic site, which is able to accommodate eleven amino acid substrates (Figure 2a).22 Inhibitors for this protease are normally large molecules able to maximize interactions in this cavity stabilizing the flexible loop on its N-terminal domain known as “the flap” through its Tyr71 and locking BACE1 in an inactive conformation.23 The central binding pockets on the active site bind hydrophobic groups such as the ones found in peptidomimetic compounds based on substrate transition-state analogues24 or other scaffolds like those showed in Figure 2b, including verubecestat,25 OM-003,26 5I3Y ligand27 and 5V0N ligand.28,29 The BACE1 pharmacophore of amidine compounds, the most advanced BACE1 inhibitors in clinical trials, highlights the importance of heteroaromatic rings linked through an amide bond to optimally occupy S1 and S3 pockets.30 Therefore our working hypothesis consisted in the use of the linkage design to build BACE1 inhibitors starting from kinase inhibitor fragments and thus, obtaining MTDs with both activities on the different AD hallmarks. Additionally, the linkage strategy also offers the possibility of connecting original scaffolds in a manner that can retain their primitive activity and interactions with the initial target.

In this work, we describe the design, synthesis, biological evaluation and suggest a binding mechanism of novel multitarget compounds with BACE1 and protein kinase inhibition activities. Furthermore, click chemistry and protein-templated synthesis using BACE1 as scaffold has been used as versatile synthetic tools. Finally, different cellular assays have also been used to confirm the polypharmacology and applicability of our MTDs.

**Results and Discussion**

**Design and evaluation of a multitarget hit as proofofconcept**

In order to test our working hypothesis, we firstly synthesized two multitarget compounds, 3 and 8, to evaluate whether linking two derivatives of a kinase inhibitor would be sufficient to gain BACE1 inhibitory activity by accommodating them into the catalytic site. Specifically, we selected the benzothiazole-based LRRK2 inhibitors as starting fragments.31 To connect the two protein kinase fragments, different linkers were assayed: an aliphatic thioether chain and the heterocyclic 1,2,3-triazole. Compounds 3 and 8 would serve as a proof of concept of activity preservation for LRRK2 inhibition and acquired BACE1 inhibition with...
molecules containing heteroaromatic rings linked through amide bonds. Synthesis of the thioether derivative 3 was performed coupling 2-aminobenzothiazole 2 with propane-1,3-dithiol in basic conditions (Scheme 1).

Derivative 8 was obtained by click-chemistry through a convergent synthesis. Azide fragment 4 was easily synthesized from 4-(azidomethyl)-benzoic acid and 2-aminobenzothiazole coupling with EDCI and DMAP to form the amide bond. Alkyne 7 was obtained after three synthetic steps. Finally, the triazole 8 was synthesized using classic copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) (Scheme 1).

After in vitro biological evaluation using human recombinant enzymes, compounds 3 and 8 showed a well-balanced inhibitory activity for the two targets. BACE1 was inhibited with IC\textsubscript{50} values of 7.14 and 3.72 µM respectively, without losing LRRK2 inhibition (IC\textsubscript{50} values of 1.88 and 1.83 µM, respectively). These compounds served as a first proof of concept for our working hypothesis. Additionally, kinetic experiments were done using human recombinant BACE1 and different concentrations of substrate and compound 8. Lineweaver-Burk double reciprocal plot (Figure S1) showed a substrate-competitive behavior in the inhibition of BACE1 by the multitarget compound 8.

Docking and binding free energy calculations showed how both inhibitors adopted similar poses within the BACE1 catalytic site (Figure 3). These large molecules established multiple hydrophobic contacts within pockets S1, S2, S3, S1' and S2' of the protease and also stabilized the flap through a π-π interaction with Tyr71. In fact, compared to the original BACE1 crystal structure, inhibitors 3 and 8 induced a closed conformation of the flap at the catalytic site, moving the Tyr71 2.7 Å and 3.6 Å, respectively.

![Figure 3. Suggested binding mode of compounds 3 and 8 within BACE1 catalytic site. Both the flap and residue Tyr71 from BACE1 crystal structure (PDB: 5I3Y) are represented in white. Compounds 3 and 8 are shown as green. π-π interactions are shown as pink dotted lines.](image)

Considering that both linkers presented similar inhibitory activity against the protease adopting a comparable binding mode, we selected the triazole 8 as our lead compound to obtain further multitarget molecules. Moreover, 1,2,3-triazoles may be considered as amide bioisosteres and are readily obtainable through click chemistry. Therefore, we considered CuAAC as the best methodology to connect diverse heterocyclic fragments due to its versatility, accessibility and functional group tolerance.

To explore the cellular activity of this MTDL regarding BACE1 inhibition, we tested the ability of compound 8 to decrease the production of toxic A\textsubscript{β}40 and A\textsubscript{β}42 in an amyloid protein precursor over-expressing human neuroblastoma SH-SY5Y cell line (SK-APP cells). Levels of A\textsubscript{β}40 and A\textsubscript{β}42, the two toxic species present in AD brains, were measured both in cellular lysates and in extracellular medium after 48 h of compound treatment. As shown in figure 4, compound 8 exerts biological action at the cellular level probably due to BACE1 inhibition, reducing the A\textsubscript{β}42 levels in the extracellular fluid and A\textsubscript{β}40 levels in extracellular fluid as well as in cellular lysates.

![Figure 4. Reduced levels of A\textsubscript{β}42 and A\textsubscript{β}40 in SK-APP cells after 48 h treatment with compound 8 at 10 µM. A) and C) extracellular fluid; B) and D) cellular lysates. Data are presented as mean ± SEM of at least three independent experiments performed in triplicate. *p<0.05; **p<0.01.](image)

With these excellent preliminary data showing that the new BACE1 in vitro activity found in the multitarget molecule is confirmed at the cellular level, we worked in exploring further compounds.

Design, synthesis and in vitro evaluation of new MTDLs: BACE1/protein kinases inhibitors.
Starting from molecules present in our in-house chemical library, we selected the most interesting heterocyclic families targeting different protein kinases. Concretely, we chose GSK3β, LRRK2, and CK1δ inhibitors, that have shown inhibitory activity in the micromolar and submicromolar range with kinase selectivity, to design new multitarget compounds.

As the synthetic pathway chosen to create the MTDL involves the 1,3-dipolar addition between azides and alkynes, we firstly examined the best positions in each heterocyclic family to introduce the alkyne and azide fragments without affecting the original biological activity of these kinase inhibitors. The most relevant leads for each family of kinase inhibitors are showed in figure 5, indicating the potential chemical modifications tolerated based on previous SAR analysis. We selected one GSK3β inhibitor, one CK1 inhibitor, and two chemically diverse LRRK2 inhibitors as starting fragments for the MTDLs synthesis.

Figure 5. Different chemical modifications tolerated by the heterocyclic kinase inhibitors available at the MBC chemical library.

After the selection of the optimal sites for modification, different alkynes and azides were synthesized taking into account commercial availability of necessary reagents and synthetic efficiency.

Overall, five different fragments were obtained; two azides derived from LRRK2 and CK1δ inhibitors and three alkynes from LRRK2, GSK3β, and CK1δ inhibitors (derivatives 4, 7, 11-13). In all cases, the synthesized fragments followed the original design incorporating the alkyne or azide at the optimal site for not interfering with the original protein kinase inhibitory activity. However, for the synthesis of the GSK3β inhibitor derivative, we encountered some synthetic difficulties since the needed reagents for the synthesis of the thiadiazolinone ring, isocyanates and isothiocyanates, are incompatible with a fair amount of chemical reactions. In this case, the strategy was subtly changed in order to obtain the fragment in an efficient and rapid manner. The aromatic ring needed for GSK3β activity was removed from the starting fragment, hypothesizing that the triazole formed after the click chemistry reaction would be positioned in a similar fashion in the final compound. In this way the linker was incorporated in the original GSK3β inhibitor structure (compound 12).

Scheme 2. i) SOCl2, THF, 70°C; ii) 2- amino-benzothiazole, 100°C, MW; iii) NaN₃, DMSO, r.t.; iv) SOCl₂, 0°C; v) 4-(prop-2-yn-1-yl)oxy)aniline, EtOH, reflux.

The other fragments (alkynes and/or azides) were synthesized without further inconvenient (Scheme 2).

The inhibitory activity of the synthesized triazoles 14-17 was evaluated against human recombinant BACE1 and their two respective kinases, in order to establish whether these new chemical entities conserved the original inhibitory activities from their fragments and gained the new one as protease inhibitor (Table 1). Given that multitarget compounds come from selective kinase inhibitors serves as a strong indicative of their selectivity against their kinase.

Table 1. Structure and activity of the 1,2,3-triazoles in their respective enzymes.

| No | Structure | LRRK2 IC₅₀ (µM) | CK1δ IC₅₀ (µM) | GSK3β IC₅₀ (µM) | BACE1 IC₅₀ (µM) |
|----|-----------|----------------|---------------|----------------|----------------|
| 14 | ![Structure](image1) | 0.82±0.14 | - | 2.36±0.2 | 3.3±0.27 |
| 15 | ![Structure](image2) | - | 1.25±0.21 | 3.85±0.3 | 2 ± 0.3 |
| 16 | ![Structure](image3) | 13.46±5.23 | 6.47±0.68 | - | 7.24±0.38 |
| 17 | ![Structure](image4) | 7.09±2.22 | - | 26%* | 2.51±0.43 |

* Enzyme inhibition at 10µM. IC₅₀ was not calculated due to poor solubility of the compound.
Results showed that most of the compounds inhibited BACE1 at the low micromolar range. Moreover, the original activity of the fragments was generally maintained, obtaining well-balanced biological activities in three different targets and pointing to good candidates to modulate the AD pathobiological pathways efficiently at the same dose.

The BACE1 inhibitory activity of the original protein kinase inhibitors used to build the multitarget compounds was also tested, showing that the initial fragments alone did not have the capacity to bind to this enzyme (Table S1). These results suggest that the starting protein kinase inhibitors lack of BACE1 activity, thus BACE1 inhibition found in compounds 14-17 was gained by the molecular volume acquired after the linkage of the two protein kinase inhibitors, confirming our initial hypothesis.

The binding mode of these new MTDs was explored with docking and free binding energy molecular dynamics studies, showing similar binding poses to compounds 3 and 8 with minor differences (Figure 6). For example, compounds 14 and 15 are smaller molecules and they preferentially occupy pockets S1, S2' and partially S1'. Compound 15 and 16 showed a shifted pose leaving pocket S3 unoccupied.

**Cellular activity of MTDs**

Cellular activity of the four new compounds (14-17) was evaluated in two different models in order to prove the beneficial effects of inhibiting BACE1 and the two different protein kinases. Firstly, the human neuroblastoma cell line transfected with the precursor amyloid protein (SK-APP cells) was used in order to confirm the ability of the MTDs treatment to modulate Aβ40 and Aβ42 levels. A preliminary cellular toxicity study of these compounds at a fixed concentration of 10 μM and after 24 and 48 h of treatment, discarded compounds 16 and 17 from the experiment as they showed a significant decrease of cell viability at 48 hours (Figure S2).

The remaining compounds, 14 and 15 continued with the cellular characterization. Derivatives 14 and 15 exhibited a significant decrease in Aβ40 levels in extracellular fluids (Figure 7A), while compound 15 was also able to decrease levels of Aβ42 both in cell lysates and extracellular media, showing their potential to interfere with toxic Aβ pathway (Figure 7C and 7D).

These decreased Aβ levels could be the consequence of a reduction in Aβ secretion from APP processing. Although intracellular Aβ40 expression was unchanged, Aβ42 levels were reduced in cellular lysates mainly after compound 15 administration. This later result might suggest that these compounds were modulating Aβ production, and the subsequent release to medium. In view of our data, we suggest that compounds 14 and 15 provoked an important reduction of Aβ-induced neurotoxicity, associated with diminished Aβ presence in the cell medium. Further dose dependent studies may be designed for more optimized lead compounds.

**Figure 6.** Binding mode of compounds 3 and 8 as well as synthesized triazoles within BACE1 catalytic site. Left panel show the crystallized structure of BACE1 in complex with 5I3Y ligand (PDB id 5I3Y).

**Figure 7.** Reduction in the levels of Aβ40 and Aβ42 in SK-APP cell line after 48 h treatment with compounds 14 and 15 at 10 μM: A) extracellular fluid; B) and D) cellular lysates. Data are presented as mean ± SEM of at least three independent experiments performed in triplicate. *p<0.05.

The protein kinase inhibition of the multitarget compounds, and specifically GSK3β, CK1δ, and LRRK2 inhibitory activities present in MTDs 14 and 15 may interfere with tau phosphorylation, other of the hallmarks of AD. To evaluate the beneficial effects of our MTDs in tau phosphorylation, the well-known model of okadaic acid (OA) in human neuroblastoma cell
In order to identify multitarget compounds with BACE1 and kinase inhibitory activity easily and effectively, we implemented the technology of in situ click chemistry. On template synthesis enables the formation of a product when reagents bind to the macromolecule acquire an optimal orientation for the chemical reaction to occur. In this manner, Huisgen cyclization can be performed in the absence of catalyst and at room temperature in a short time frame. We envisioned that by leveraging this methodology we could quickly identify BACE1 binders starting from kinase inhibitor fragments prior to synthesizing them in a bigger scale and thus saving resources.

Azide 4 and alkyne 7 were chosen to test the in situ click reaction with BACE1. Derivatives 4 and 7 were mixed in presence or absence of BACE1 in aqueous buffer and the mixtures were analyzed by LC/MS-SIM. Concentration of the enzyme (0.25, 0.5, and 1 µM) and reagents (10, 50, 100, and 200 µM), temperature (20 and 37°C), time (24 and 48 h) and buffer (phosphate-buffered saline (PBS) pH 7.0 or sodium acetate pH 4.5) were screened in independent experiments performed in triplicate. (⁎p<0.05, **p<0.005, ***p<0.001 vs treatment with OA only).

On target BACE1 directed synthesis

In order to identify multitarget compounds with BACE1 and kinase inhibitory activity easily and effectively, we implemented the technology of in situ click chemistry. On template synthesis enables the formation of a product when reagents bind to the macromolecule acquire an optimal orientation for the chemical reaction to occur. In this manner, Huisgen cyclization can be performed in the absence of catalyst and at room temperature in a short time frame. We envisioned that by leveraging this methodology we could quickly identify BACE1 binders starting from kinase inhibitor fragments prior to synthesizing them in a bigger scale and thus saving resources.

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To implement this methodology, other building blocks starting from different known protein kinase inhibitors were designed, inspired by kinase inhibitors in our chemical library. Thus, five new alkynes derived from inhibitors of CK1ε and GSK3β together with a new LRRK2 azide were synthesized following previous described experimental conditions (Scheme 4).

In total, three different azides (4, 11, and 27) and eight diverse alkynes (7, 12, 13, 20-23, and 25), with inhibitory activities in their respective protein kinases LRRK2, CK1ε and GSK3β, were...
combined in order to further select multitarget compounds (Table 2).

Table 2. In situ click chemistry building blocks with their inhibitory kinase properties.

| Kinase | Building Block | Inhibitory Kinase Properties |
|--------|----------------|-----------------------------|
| LRRK2  | 27             |                             |
| CK1δ   | 11             |                             |
| GSK3β  | 12             |                             |

*In situ* click chemistry experiments were set incubating both fragments in the presence or absence of BACE1 and the reaction was analyzed by SIM LC-MS. A total of nineteen independent reactions were set, from which we obtained eleven positive hits, observing that without the presence of BACE1 the subsequent triazole MTD was not being formed (Figure 10 and S3-S6).

Figure 10. Hits obtained by *in situ* click chemistry reaction with BACE1 after incubation with the corresponding building blocks for 24 hours. Compounds synthetized to confirm the hypothesis are numbered above the bars: 28-31 positive hits and 32-33 negative hits. Data presented here is a result of two independent runs.

To fully confirm that hits obtained in this protein template synthesis correspond to BACE1 inhibitors, we synthetized four different MTDs based in the availability of starting building blocks (compounds 28-31). Furthermore, to control the reaction selectivity, two derivatives more (compounds 32 and 33) were also prepared as negative controls (Scheme 5). In all cases, 1,2,3-disubstituted triazoles were obtained using classical CuAAC.

Inhibitory activity of compounds 28-33 was tested on BACE1 and the corresponding protein kinases (Table 3). Results confirmed how hits selected by BACE1-templated synthesis show inhibitory activity on this protease while negative controls lack this activity. In general, protein kinase inhibition profile was maintained in the multitarget compounds in a well-balanced manner.

Table 3. Structure and activity of the new 1,2,3-triazoles in their respective enzymes.

| Structure | LRRK2 IC₅₀ (μM) | CK1δ IC₅₀ (μM) | GSK3β IC₅₀ (μM) | BACE1 IC₅₀ (μM) |
|-----------|-----------------|----------------|-----------------|-----------------|
| 28        | 0.71 ± 0.26     | 0.40 ± 0.06    | 7.21 ± 0.35     |
| 29        | 0.44 ± 0.10     | 12.58 ± 0.70   | -               | 11.00 ± 1.36    |
| 30        | 3.19 ± 1.01     | 30%*           | -               | 8.57 ± 0.45     |
| 31        | -               | 5.40 ± 0.91    | 26%*            | 6.76 ± 0.61     |
The in silico binding mode of the active triazoles on BACE1 was also studied, and it resembled those of compounds 3 and 8 (Figure S7). A detailed study of predicted binding energies (MMGBSA values) and interactions with key BACE1 residues have been done with MTDs and the initial fragments (Table S2), showing lower energy values and different interactions for MTDs BACE1 inhibitors (such as hydrophobic, hydrogen bonds and π-π contacts with Try71 residue, or cation-π interactions with Arg128 that their corresponding starting fragments. Finally, a molecular dynamics study performed on MTD compounds 14 and 32, active and inactive on BACE1 respectively, together with their starting fragment MBC-2137 show clearly how the active compound 14 present lower ΔGbind binding energy than the inactive 32 as well as the fragment MBC-2137 (Figure 11). This analysis indicates that MTD14 presents more favorable interactions making its affinity for BACE1 higher, which is directly related to the inhibitory activity of BACE1 (Figures S8-S13).

![Figure 11. ΔGbind binding energy calculated using MMGBSA along the simulations. The ΔGbind average ± standard deviation (kcal/mol) is displayed in the legend.](image)

**Conclusion and future perspectives**

In the recent decade, an unprecedented increase of polypharmacology and multitarget directed ligands (MTDs) design strategies as the new chance to develop effective treatments for multifactorial severe diseases has been noted. We have here designed and synthetized new multitarget compounds that maintained their original kinase inhibitory activity adding a new BACE1 inhibitory effect. Therefore, we describe new MTDs with great potential to treat complex diseases such as AD where Aβ pathology and aberrant phosphorylation events on tau protein are key events. The suggested binding mode of these compounds to BACE1 was simulated by molecular dynamic calculations showing how the compounds fit in the catalytic cavity closing it by direct interaction with the protein flap.

The new BACE1 inhibitory activity was further confirmed in a cellular model where these MTDs were able to reduce Aβ42 and Aβ1-40 levels. Additional cellular assays were performed to test the potential of these multitarget compounds for protecting neural cells in the okadaic acid-induced cell death model. MTDs showed neuroprotective potential against the tau phosphorylation toxic effect with similar or improved potency than the simultaneous treatment with an equimolar mixture of their protein kinase inhibitor precursors. Besides we have demonstrated for the first time, how BACE1 can be used as template to select the artificial membrane permeability assay (PAMPA) to predict the in vitro permeability (P₂) by passive diffusion (Table S3 and Figure S14). While some compounds were insoluble in the conditions of the experiment (8, 17 and 31), brain permeation prediction was calculated for MTDs derivatives 14-16 and 28-30. Compounds 15 and 30 showed apparent permeability values compatible with a positive brain penetration while compound 16 remains in the uncertainty region of prediction (CNS+/CNS-). Compounds 14 and 29 were not detected in the acceptor well by HPLC-MS and therefore experimental P₂ was assumed as 0 (Figure 12). These data confirm the difficulty of MTDs to maintain good pharmacokinetic profiles but show two of the new MTDs as potential brain penetrant compounds and therefore suitable agents to treat AD and other dementias. Further studies aiming to improve brain penetration of these compounds using PLGA nanocapsulation are ongoing.
fragments that may be exploited to obtain these and others interesting MTDLs. Additionally, we experimentally predicted that some of these MTDLs may penetrate into the brain by passive diffusion using PAMPA methodology.

Our strategy based on the interaction with the long catalytic site of BACE1 starting from individual protein kinase inhibitors to obtain new MTDLs has been confirmed. The cellular potency of the compounds has demonstrated how exploring this strategy could lead to promising compounds for the treatment of neurodegenerative diseases and specially Alzheimer’s disease. In vivo evaluation of the brain penetrating multitarget compounds or the nanoencapsulated ones in a AD model will finally confirm the translational potential of these compounds.

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Using different small molecules targeting protein kinases and BACE1 templated synthesis, we have obtained well-balanced multitarget compounds. They have three different biological activities and may be a promising therapeutic approach for the treatment of complex diseases such as AD.

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