Rational design of small molecules able to inhibit \( \alpha \)-synuclein amyloid aggregation for the treatment of Parkinson’s disease

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ABSTRACT
Parkinson’s disease is one of the most common neurodegenerative disorders in elderly age. One of the mechanisms involved in the neurodegeneration appears related to the aggregation of the presynaptic protein alpha synuclein (\( \alpha \)-syn) into toxic oligomers and fibrils. To date, no highly effective treatment is currently available; therefore, there is an increasing interest in the search of new therapeutic tools. The modulation of \( \alpha \)-syn aggregation represents an emergent and promising disease-modifying strategy for reducing or blocking the neurodegenerative process. Herein, by combining \textit{in silico} and \textit{in vitro} screenings we initially identified 3-(cinnamylsulfanyl)-5-(4-pyridinyl)-1,2,4-triazol-4-amine (3) as \( \alpha \)-syn aggregation inhibitor that was then considered a promising hit for the further design of a new series of small molecules. Therefore, we rationally designed new hit-derivatives that were synthesised and evaluated by biological assays. Lastly, the binding mode of the newer inhibitors was predicted by docking studies.

1. Introduction
Parkinson’s disease (PD) is a neurodegenerative disorder characterised by the loss of dopaminergic neurons in the substantia nigra of the brain. These cells are involved in the production of the neurotransmitter dopamine which regulates the muscular movements\(^1\). Typical manifestations of PD include motor symptoms due to the dopaminergic loss, like bradykinesia, rigidity, postural instability and rest tremor\(^2\). Additionally, non-motor features such as olfactory dysfunction, constipation, cognitive impairments, depression and sleep disorders can occur; these further symptoms are due to the implication of the neurodegenerative process in other areas of the peripheral and central nervous system\(^3\). The hallmark of PD is represented by the presence of neuronal inclusions, termed Lews Bodies, mainly composed of aggregates of misfolded alpha synuclein (\( \alpha \)-syn)\(^4\). \( \alpha \)-Syn is a 140 aa presynaptic protein which regulates the release of neurotransmitters from the synaptic vesicles\(^5\). From a structural point of view, \( \alpha \)-syn is organised in three different regions: the N-terminal domain (aa 1-60), the central NAC domain (aa 61-95) and the C-terminal domain (aa 96-140)\(^6\). In its monomeric soluble form, \( \alpha \)-syn assumes an alpha helical conformation upon interaction with phospholipids,\(^7\) while in the pathological misfolded state, it aggregates into amyloid fibrils composed by parallel hydrogen bonded \( \beta \)-sheets\(^8,9\). The formation of these aggregates causes cytotoxicity through lipid membrane permeabilisation, mitochondrial damage and oxidative stress\(^10\). Another relevant mechanism that contributes to the propagation of neurodegeneration is the prion-like spread of \( \alpha \)-syn aggregates. Indeed, experimental studies revealed that the injection of \( \alpha \)-syn inclusions in an animal’s brain promotes the formation of cellular inclusions at the injection site from where they can spread in other brain regions\(^11\). To date, the therapies available for the treatment of PD are addressed to reduce the motor symptoms and include the administration of drugs able to restore the level of dopamine. Among them the most used is L-Dopa, which acts as a prodrug being converted in dopamine in the brain\(^1,12\). Other dopaminergic drugs used for the treatment of PD are
dopamine agonists like ropinirole or rotigotine, monoamine oxidase B (MAO-B) inhibitors such as rasagiline and selegiline and catechol-O-methyltransferase (COMT) inhibitors such as tolcapone and entacapone which inhibit the enzymes responsible of dopamine metabolism. Unfortunately, the use of these drugs induces unwanted side effects such as dyskinesia, dizziness, headaches, nausea and somnolence. More serious problems like hallucinations, confusion and impulse control disorders are often associated with the assumption of dopamine agonists. Furthermore, these therapies lose their efficacy as the disease progresses and are unable to block or reduce the neurodegenerative process. In the last decade, several efforts have been made to find a disease modifying strategy to halt or slow the neurodegeneration.

In this work, we applied a pharmacophore-based virtual screening approach as effective tool to discover novel a-syn aggregation inhibitors. Firstly, we developed a computational model that was subsequently combined with in vitro experiments to test their ability to reduce a-syn aggregation; as result we discovered a small molecule as promising inhibitor, which was used as lead compound for the development of a further series of compounds. Then, the designed molecules were synthesised, tested in vitro and studied to decipher the putative binding mode by molecular docking simulation.

2. Materials and Methods

2.1. Pharmacophore modelling and virtual screening

LigandScout V4.4 was used for the pharmacophore generation and the virtual screening experiments. Three small molecules able to bind to the N-terminal region of a-syn have been selected from literature and used as training set. A shared-feature pharmacophore model was created applying the default settings. All virtual screening runs were performed by setting the option “Get best matching conformation” as retrieval mode.

2.2. Chemistry

All reagents were used without further purification and bought from common commercial suppliers. Melting points were determined on a Carlo Erba Modell1106-Elemental Analyser we determined the purity of synthesised compounds; the results confirmed a ± 95% purity. Merck Silica Gel 60 F254 plates were used for analytical TLC (Merck KGaA, Darmstadt, Germany). By combustion analysis (C, H, N) carried out on a Carlo Erba Model1106-Elemental Analyser we determined the purity of synthesised compounds; the results confirmed a ± 95% purity. All reagents were used without further purification and bought from common commercial suppliers.

2.2.3. General procedure for the synthesis of pyridinyl-triazole derivatives 5-11

The 4-amino-5-((4-pyridinyl)-4H-1,2,4-triazole-3-thiol 12 (200 mg, 1.03 mmol) was dissolved in a mixture of NaOH (41 mg, 1.03 mmol) and MeOH (5 ml); after complete dissolution, the suitable benzyl bromide derivative (194.7 mg, 1.03 mmol) was added. The reaction mixture was stirred for 15 min at room temperature for desired compounds 5 and 7-11, whereas for desired compound 6 the reaction time was 120 min. The resulting solid residue was washed with water, dried and recrystallized from ethanol to provide pure desired final pyridyl-triazole derivatives 5-11. For all synthesised compounds the registered CAS numbers have been already assigned; However, the synthetic procedure, chemical and structural characterisation are not available in literature with exception of compound 5, for which the structural characterisation is an agreement with literature.

2.2.3.1. 3-((Phenethylthio)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-amine (6). CAS number: 691366-30-0. Yield 12%. Yellow solid. M.p. 168–169°C. 1H NMR (DMSO-d6): δ 3.04 (t, 2H, J = 7.74 Hz, CH2); 3.44 (t, 2H, J = 7.74 Hz, CH2); 6.19 (s, 2H, NH2); 7.21–7.82 (m, 9H, ArH) Anal. for C13H13NS: C, 60.58%; H, 5.08%; N, 23.55%. Found C, 60.48%; H, 5.33%; N, 23.43%.

2.2.3.2. 3-((4-Fluorobenzyl)thio)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-amine (7). CAS number: 575460-68-3. Yield 39%. Yellow solid. M.p. 186–187°C. 1H NMR (DMSO-d6): δ 4.44 (s, 2H, CH2); 6.21 (s, 2H, NH2); 7.13–8.71 (m, 8H, ArH). 13C NMR (DMSO-d6): δ 34.0; 115.1 (d, 3JCF = 19.1 Hz); 115.3 (d, 3JCF = 19.1 Hz); 121.2; 121.3; 131.0 (d, 3JCF = 8.6 Hz); 131.1 (d, 3JCF = 8.6 Hz); 133.8 (d, 3JCF = 2.9 Hz) 134.0; 150.1; 152.0; 154.5; 161.4 (d, 3JCF = 243.2 Hz). Anal. for C14H12FN4S: C, 55.80%; H, 4.01%; N, 23.24%. Found C, 55.68%; H, 4.31%; N, 23.44%.

2.2.3.3. 3-((3-Fluorobenzyl)thio)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-amine (8). CAS number: 674812-86-3. Yield 52%. Yellow solid. M.p. 164–165°C. 1H NMR (DMSO-d6): δ 4.45 (s, 2H, CH2); 6.25 (s, 2H, NH2); 7.06–8.72 (m, 8H, ArH). 13C NMR (DMSO-d6): δ 34.6; 114.6 (d, 3JCF = 20.9 Hz); 116.3 (d, 3JCF = 21.0 Hz); 121.7; 121.8; 125.6 (d, 3JCF = 2.7 Hz); 130.8 (d, 3JCF = 8.2 Hz); 134.4; 141.0 (d, 3JCF = 8.2 Hz); 150.5; 152.5; 154.8; 162.4 (d, 3JCF = 243.4 Hz). Anal. for C15H15N5S: C, 60.58%; H, 4.31%; N, 23.24%. Found C, 55.89%; H, 4.00%; N, 23.44%.

2.2.3.4. 3-((3-Methylbenzyl)thio)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-amine (9). CAS number: 676147-32-3. Yield 13%. Yellow solid. M.p. 180–181°C. 1H NMR (DMSO-d6); δ 2.25 (s, 3H, CH3); 4.40 (s, 2H, CH2); 6.19 (s, 2H, NH2); 7.10–8.71 (m, 8H, ArH). 13C NMR (DMSO-d6): δ 21.0; 35.2; 110.0; 121.7; 121.8; 129.6 134.4; 137.1; 141.0; 150.4; 150.7; 152.4; 155.1. Anal. for C15H15N5S: C, 60.58%; H, 5.08%; N, 23.55%. Found C, 60.23%; H, 5.26%; N, 23.40%.

2.2.3.5. 3-((3-Methoxybenzyl)thio)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-amine (10). CAS number: 676548-28-0. Yield 16%. Whithis solid. M.p 175–176°C. 1H NMR (DMSO-d6): δ 2.26 (s, 3H, CH3); 4.40 (s, 2H, CH2); 6.19 (s, 2H, NH2); 7.07–8.71 (m, 8H, ArH). 13C NMR (DMSO-d6): δ 21.3; 35.4; 121.7; 126.7; 128.5; 128.8; 130.0; 134.4; 137.6; 138.0; 150.6; 152.4; 155.1. Anal. for C14H12FNS: C, 60.58%; H, 5.08%; N, 23.55%. Found C, 60.70%; H, 5.01%; N, 23.32%.

2.2.3.6. 3-(4-Chlorobenzyl)thio)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-amine (11). CAS number: 901093-20-7. Yield 39%. Yellow solid. M.p 198–199°C. 1H NMR (DMSO-d6): δ 4.44 (s, 2H, CH2); 6.22 (s, 2H, NH2); 7.37–8.70 (m, 8H, ArH). 13C NMR (DMSO-d6): δ 34.4; 121.7; 121.8; 128.61; 129.0; 131.2; 131.5; 132.4; 134.4; 137.2; 150.4;
150.7; 152.5; 154.8. Anal. for C_{14}H_{12}ClN_{5}S: C, 52.91%; H, 3.81%; N, 23.04%. Found C, 52.66%; H, 3.78%; N, 23.36%.

2.2.2.3. Alpha synuclein aggregation and inhibition in vitro assays

WT α-Syn was expressed and purified as previously described. The resultant protein was kept lyophilised at −80 °C. Lyophilised protein was delicately resuspended in sterile PBS 1X and filtered through 0.22-μm membrane to eliminate small aggregates. Aggregation assays were performed as previously described for ZPD-2 and SynuClean-D, the latter used as reference compound for kinetic studies. Briefly, 70 μM of α-Syn, in a final volume of 150 μL, was placed in a sealed 96-well plate, which also contains 40 μM Th-T in PBS 1X, a 1/8” diameter Teflon polyball (Polysciences Europe GmbH, Eppelheim, Germany) and 100 μM of the different compounds or DMSO (in control samples). The plates were fixed and 100 rpm. Th-T fluorescence emission was measured every 2 h in a Victor3.0 Multilabel Reader (PerkinElmer, Waltham, Massachusetts, USA) by exciting through a 430–450 nm filter and collecting with a 480–510 filter. Triplicates were performed and the kinetics were fitted with the following equation:

\[ \alpha = 1 - \frac{1}{k_0(e^{kt} - 1) + 1} \]  

In which \( k_0 \) and \( k_a \) represent the homogeneous nucleation rate constant and the secondary rate constant (it means, fibril elongation and secondary nucleation), respectively.

Light-scattering measurements were performed in a Cary Eclipse Fluorescence Spectrophotometer (Agilent, Santa Clara, California, USA). 80 μL of end-point aggregates was placed into a quartz cuvette and excited at 300 nm, the 90° emission was thus collected between 280 and 360 nm.

For transmission electron microscopy (TEM) assays, endpoint treated and untreated aggregates diluted 1:10 in PBS 1X. Samples were then softly sonicated for 5 min and 5 μL of the resultant mixture were placed on a carbon-coated copper grid for 5 min. The grids were dried with filter paper to remove the excess and washed twice in miliQ water, whose excess was also removed. Finally, 5 μL of 2% (w/v) uranyl acetate solution was added and left incubate for 2 min. As previously, the excess of uranyl acetate was removed, and grids were left to air-dry for 10 min. Images were obtained using a Transmission Electron Microscopy Jeol 1400 (Peabody, Massachusetts, USA) operating at an accelerating voltage of 120 kV. A minimum of 30 fields were screened per sample, in order to collect representative images.

2.4. Docking studies

Docking studies were performed by Autodock4.2 software by using the solid-state NMR of alpha-synuclein fibrils retrieved from RCSB Protein Data Bank (PDB code 2N0A). The structure is characterised by a central part with a greek-key topology and terminal flexible loops. A grid box with dimension of 126 × 126 × 126 Å³ and centre \( x = 97.487, y = 148.695 \) and \( z = -34,111 \), was applied in order to include the aminoacid residues of the N-terminal region discarding the ones present in the unstructured flexible loops. Ligand structures were constructed by Vega ZZ software and energy minimised by following a conjugate gradient minimisation by AMMP calculation as implemented in VEGAZZ program. The Lamarckian Genetic Algorithm was used to calculate 10 protein – ligand binding poses for each compound by using the default settings. The highest scored docking pose was chosen for analysis and representation. PyMOL software was used to visualise docking results while the analysis of the putative ligand–protein interactions was performed by Discovery Studio Visualiser.

3. Results and discussion

3.1. Pharmacophore model generation, virtual screening and preliminary biological assay

With the aim to find new molecules able to inhibit α-syn aggregation, we used a computational approach to generate a ligand-based pharmacophore model by means of the software LigandScout.

To build the model, we used a training set (TS) including α-syn aggregation inhibitors that target the N-terminal portion of the protein. Specifically, we selected three small molecules from literature belonging to different chemotypes as polyphenolic and non-polyphenolic inhibitors (Table 1) for which the binding interaction to N-terminal portion of α-syn is corroborated by experimental data.

In particular, we considered only the common features for all the selected molecules of the TS for the model construction generating shared feature pharmacophore models.

As result, we obtained ten pharmacophore hypotheses and selected the hypothesis possessing the highest score to perform virtual screening studies. As displayed in Figure 1, the above-mentioned pharmacophore model consisted of five features: (i) two hydrogen bond acceptors, (ii) two hydrophobic features, (iii) one aromatic feature and 45 excluded volumes as forbidden areas.

| NAME | STRUCTURE |
|------|-----------|
| Congo Red | ![Congo Red Structure](image) |
| Lacmoid | ![Lacmoid Structure](image) |
| Rosmarinic Acid | ![Rosmarinic Acid Structure](image) |
The obtained pharmacophore model was used as a query to screen two three-dimensional databases containing small molecules belonging to different structural chemotypes: (i) the in-house 3D database CHIME collecting 1329 molecules designed and synthesised by our group over the years, (ii) the MyriaScreen Diversity Library II, consisting of 10,000 compounds with druglike properties (https://www.sigmaaldrich.com/chemistry/chemistry-services/high-throughput-screening/screening-compounds.html).

From CHIME, the virtual screening led to the identification of three hits 1a–c (Figure 2(A)). Considering that these molecules shared a very similar structure, we decided to select only 2-[4-[(4-fluorophenyl)methyl]-1-piperidyl]-1-(6-methoxy-1H-indol-3yl)ethenone (1a) displaying the highest pharmacophore fit-score as representative inhibitor filtered through virtual screening on CHIME database (Figure 2(B)).

The virtual screening on MyriaScreen Diversity Library II resulted in 113 hits (see supporting material). Among them, we selected three compounds (Figure 3) such as 3-[[5-[(4-methoxyphenyl)methylsulfanyl]-4-methyl-1,2,4-triazol-3-yl]pyridine (2), 3-(cinnamylsulfanyl)-5-(4-pyridinyl)-1,2,4-triazol-4-amine (3) and 3-(3-chloro-4-fluoro-anilino)-1-(2-naphthyl)propan-1-one (4); the selection of the compounds 2–4 was made basing on the pharmacophore-fit score value (>57) and the commercial availability from the supplier.
All selected compounds 1–4 proved to respect the Lipinski’s rule and no PAINS were in silico predicted by the online tool SwissAdme platform [http://www.swissadme.ch/]. To evaluate the ability to inhibit 

\( \alpha \)-syn aggregation, we got compounds 1–4 as follows. Compound 1 was readily synthesised in our laboratory following a previously optimised synthetic approach;\(^{28} \) while the three commercially available compounds 2–4 were purchased from Sigma Aldrich as supplier of bioactive molecules [https://www.sigmaaldrich.com/italy.html].

The anti-aggregation potential of selected compounds (1–4) was evaluated by a robust protocol previously applied in the identification of strong inhibitors of 

\( \alpha \)-syn aggregation\(^{16,24} \) like SynuClean-D, a well-known inhibitor of \( \alpha \)-synuclein (\( \alpha \)-Syn) aggregation\(^{24} \) that was used as reference compound in the same test. The presence of 100 \( \mu \)M of the compounds during the incubation of 70 \( \mu \)M of \( \alpha \)-Syn, reduced Thioflavin-T (Th-T) fluorescence emission up to a 10, 26, 31 and 10% for compound 1, compound 2, compound 3 and compound 4, respectively. As result, all tested compounds impacted the kinetic constants (see Figure 4(A)). Particularly, the compound 2 reduced the homogeneous nucleation rate constant \( (k_0 = 0.01591) \) when compared to untreated sample \( (k_0 = 0.03234) \). In contrast, compound 3 reduced the autocatalytic rate constant \( (k_a = 0.1892 \text{ h}^{-1}) \), compared to the control \( (k_a = 0.2037 \text{ h}^{-1}) \). The compounds 1 and 4 did not impact significantly the aggregation rate constants.

To confirm these results light-scattering measurements at 300 nm were performed at the end-point of the reaction, thus revealing a reduction of 13 and 43% of aggregates in the presence of compounds 2 and 3, respectively.

To further assess the impact of the best candidate 3 on \( \alpha \)-syn aggregation, end-point samples were analysed by transmission electron microscopy (TEM). Notably, TEM images certified a significant reduction in the amount of fibrillar material in the presence of compound 3 (Figure 4(D)), when compared to untreated samples (Figure 4(C)).

Therefore, this first screening highlighted that the best in vitro results were obtained for compound 3. This compound is characterised by the presence of a pyridinyl-triazole moiety similarly to compound 2, in which the nitrogen of the triazole ring presents a methyl substituent while an amino group is present in compound 3 in the same position. Considering the obtained results, we can speculate that the amino group could establish fundamental interaction with the protein important for the inhibition of \( \alpha \)-syn aggregation.

\[
\text{3.2. Design and synthesis of new small molecules as derivatives of compound 3}
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Based on the above reported results of biological screening, we chose compound 3 as promising lead compound for the rational design of a further series of pyridinyl-triazole derivatives 5–11 (Scheme 1) for which a very simple synthetic approach might be carried out.

In particular, we chose to maintain the pyridinyl-triazole moiety of inhibitor 3 and reduce the length of the linker between the sulphur atom and the phenyl ring. Moreover, we planned to extend the series by introducing selected substituents in meta and in para positions of phenyl ring. In detail, we introduced F, Cl or CH\(_3\) substituents in order to preliminary probe the influence of electron withdrawing group (EWG) or electron donating group (EDG) on the aggregation of \( \alpha \)-syn. For each designed derivative we evaluated the adherence to the Lipinski’s rule and the absence of PAINS in silico by using SwissADME. Scheme 2 shows the synthetic procedure to obtain compounds 5–11 starting from the 4-amino-5-(4-pyridinyl)-4H-1,2,4-triazole-3-thiol 12, that was coupled with the appropriate benzyl bromide in alkaline medium at room temperature.

Figure 3. (A) Chemical structures of compounds 2, 3 and 4. (B) Alignment of each of the reported hits (represented by pink (2), orange (3) and yellow (4) sticks) with the pharmacophore model.
3.3. Screening of the activity of the new derivatives 5–11

The synthesised derivatives were tested in order to study their ability to reduce \( \alpha \)-syn aggregation \textit{in vitro} by using the method described above. Incubation of 70 \( \mu \)M \( \alpha \)-Syn in the presence of 100 \( \mu \)M of the different compounds, followed by Th-T fluorescence measurements at the end of the reaction indicated that 5, 8, 9 and 11 could reduce the aggregation up to 33, 15, 19 and 29\%, respectively (Figure 5(A)). Light-scattering measurements at 300 nm indicated that 5, 6, 7, 8, 9 and 10 reduced the amount of aggregates up to a 23, 16, 26, 14, 24, 17\%, respectively (Figure 5(B)). However, TEM analysis indicated that only 5 (Figure 5(E)), 8 (Figure 5(F)), 9 (Figure 5(G)) and 11 (Figure 5(H)) reduced the aggregation.
number of amyloid fibrils when compared to control samples (Figure 5(D)), in good agreement with the kinetic analysis.

### 3.4. Docking studies

In order to ascertain the binding mode of pyridinyl-triazole derivatives 3, 5, 8, 9 and 11 that demonstrated the ability to reduce α-syn aggregation in vitro, we carried out molecular docking simulation by means of Autodock 4.2 suite; the computational studies were based on NMR structure of α-syn (PDB code 2N0A) as structural coordinates.

Considering that in our above reported molecular modelling studies we selected polyphenolic and non-polyphenolic inhibitors (see Table 1) that bind the N-terminal region of α-syn, we hypothesised that also derivatives 3, 5, 8, 9 and 11 could interact with same portion of the protein. For this reason, we defined this region as the search space for the docking simulation (see Material and Methods section). The docking results confirmed that all the tested compounds bind in the same site of α-syn located between the N-terminal and the NAC domains of the protein and lined by Ala53, Val55, Thr59, Glu61, Thr72, Gly73 and Val74 (Figure 6).
Notably, in a recent work, Pujols and co-workers identified the same portion of the protein as binding site of their inhibitor 2-hydroxy-5-nitro-6-(3-nitrophenyl)-4-(trifluoromethyl)nicotinonitrile through a induced-fit docking simulation\(^{24}\); therefore, these evidences further supported the hypothesis that this region could represent a binding site for small molecules on α-syn fibrils.

In detail, compound 3 could bind to α-syn by establishing a salt bridge between the amino group and Glu61 and π-anionic interaction between the pyridine ring and Glu61B. The cinnamyl moiety is oriented towards Ala53, Gly73 and Val74 and might engage hydrophobic interaction with Ala53. Furthermore, van der Waals interactions with Val55, Thr59, Thr72, Gly73 and Val74 could be observed (Figure 7).

In Figure 8, the binding mode of compounds 5, 8, 9 and 11 is displayed. The new derivatives showed a slight different binding orientation in comparison to the parent compound 3, probably due to the shorter and more flexible linker between the sulphur atom and the aromatic ring.

In particular, all the inhibitors could interact with α-syn by a crucial salt bridge between the amino group and the side chain of Glu61. Moreover, for inhibitors 5, 8, 9 a π-anion interaction was observed between the pyridine ring and Glu61B.

Additionally, compound 5 might form a hydrogen bond with Thr72C through the sulphur atom and π-sigma interaction with Thr72C (Figure 8, Panel A). Concerning compound 8, it might establish a halogen bond between the fluorine atom and Thr72D (Figure 8, Panel B). Instead, 9 could engage π-sigma interaction with Thr72C (Figure 8, Panel C). Finally, compound 11 might establish van der Waals interactions with Val55C (Figure 8, Panel D).

4. Conclusion

The search for a cure of PD represents an important challenge in the pharmaceutical research field. The inhibition of α-syn aggregation has emerged as promising new therapeutic strategy for the treatment of PD. In this work, we described the generation of a ligand-based pharmacophore model, which was used as query to screen two chemical libraries. The hits selected from the virtual
screening were tested in vitro to probe their ability to inhibit \( \alpha \)-syn aggregation, resulting in the identification of the new hit 3. Structural modifications were carried out on compound 3 obtaining four interesting derivatives. Finally, the binding mode of this new class of inhibitors on \( \alpha \)-syn was investigated by molecular docking simulation. All the information gained from these studies could be useful for the design of novel inhibitors \( \alpha \)-syn aggregation inhibitors bearing a pyridinyl-triazole scaffold.

**Disclosure statement**

The authors declare that there is no conflict of interest.

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