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Design and Neuroprotective Screening of Imidazole Derivatives As Multi-Target Agents for Alzheimer's Disease

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ABSTRACT

Alzheimer disease (AD) is a multifactorial, complicated pathology that requires therapeutic treatments that extend beyond the single-target approach. The present study used a rational multi-target directed ligand (MTDL) to create new imidazole-based hybrids that would simultaneously target the primary and secondary pathological processes of AD. On the basis of in silico target validation, molecular docking and pharmacophoremodeling, a targeted library of 25 compounds was prepared using a hybrid pharmacophore and bioisosteric replacement method. The main structural differences were considered in the imidazole core, linker (piperazine, amide, oxime ether) and aromatic (phenyl, benzimidazole) cap. Functional activities such as microtubule stabilization, anti-inflammatory, antioxidant and metal chelation and the primary target inhibition (acetylcholinesterase/AChE, butyrylcholinesterase/BuChE, b-secretase/BACE-1) were selectively filtered in the compounds. IMZ-15 with a benzimidazole cap, an oxime ether linker, and -CF3 substituent became the lead

candidate. It showed potent and balanced inhibition of AChE (IC50 = 65.4 nM) and BACE-1 (IC50 = 0.52 uM), marked microtubule-stabilizing action, strong anti-inflammatory and metal-chelating effect, and outstanding neuroprotective effects against oxidative and amyloid- beta insults. More importantly, IMZ-15 was not cytotoxic at 100 uM, which shows a large therapeutic index. These findings support the use of the MTDL strategy, which makes IMZ-15 a highly prospects and multi-functional neuroprotective agent that is worth investigating to treat Alzheimer disease.

Key words: Alzheimer's disease, Multi-target directed ligands (MTDLs), Imidazole derivatives, Neuroprotection, Acetylcholinesterase inhibition.

INTRODUCITON

A multi-target directed ligand approach is essential to the multi-faced pathology of complex neurodegenerative diseases, including Alzheimer disease, and its rational design of the novel therapeutic agents. The first and most important stage of the study will be thorough in silico target identification and validation, which is used to select and prioritize biological targets rationally in light of their known or putative role in illness pathogenesis. This method of computation will start with an extensive literature review aimed at outlining the primary and secondary pathological cascades in AD[1]. The key ones are the ones that are directly and centrally engaged in the fundamental hallmarks of pathology. The most important ones include acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and beta-secretase 1 (BACE-1). The hydrolysis of the neurotransmitter acetylcholine is carried out by the cholinesterases, and the inhibition of these enzymes continues to be a key part of the symptomatic care of AD, which increases the cholinergic neurotransmission in the neurons that survive. Notably, AChE, uniquely, plays a dual (non-catalytic) role in enhancing amyloid-beta (Ab) aggregation by its peripheral anionic site (PAS), which is why dual-binding site AChE inhibitors hold promise as a potential approach to enhance cognition and reduce amyloid toxicity[2]. At the same time, BACE-1 is the rate-limiting enzyme in the amyloidogenic processing of the amyloid precursor protein (APP), which results in the production of neurotoxic Ab peptides. BACE-1 inhibition is a direct disease-modifying agent that tries to decrease the synthesis of the pathogenic peptides at the location. Moreover, as neuronal cytoskeletal integrity is a critical factor, strategies to facilitate microtubule stabilization, similar to the activity of the natural product paclitaxel but modified to neuronal tau pathology, are explored as the main target to counteract the hyperphosphorylation of tau and the subsequent degradation of axonal transport[3]. Nevertheless, interfering with these major pathways is in most cases not enough since the development of AD is driven by a group of downstream and parallel pathological processes. Thus, an effective target profile should include significant secondary targets that are present in the related neurotoxic cascades. These mostly contain targets in anti-inflammatory, antioxidant and metal homeostasis pathways. Activated glial cells cause neuroinflammation that worsens neuronal damage via release of pro-inflammatory cytokines and reactive oxygen/nitrogen species. In this way, it is proven that such enzymes as cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX), and inducible nitric oxide synthase (iNOS) can be considered important secondary targets. The dual COX/5-LOX inhibition is especially desired to offer the anti-inflammatory effects, and even avoid the cardiovascular risks related to selective COX-2 inhibitors. Since the AD brain has a serious oxidative stress, the targets of the endogenous antioxidant response system, including the transcription factor Nrf2, would also be held to increase the mechanisms of cell protection[4]. Also, the biometaldyshomeostasis of copper, zinc, and iron in the brain adds to the oxidative stress and the Ab aggregation. Consequently, the ability of a candidate molecule to serve as a moderate metal chelator, reallocating and not depriving these metals is found as a beneficial secondary pharmacological attribute to monopolize metal-mediated Aboligomerization and radical creation. After identifying the target, the study then uses advanced computational chemistry in order to gain insight into the interactions at the atomic scale as well as inform the design of new chemical species. Simulations of molecular docking are the basis of this stage. It is carried out by means of computational prediction of the desired orientation (pose) and affinity of a small molecule ligand upon binding to an active site of a target protein[5].

Docking studies are carefully performed to study both catalytic anionic site (CAS) and the peripheral anionic site (PAS) of a primary target such as AChE. It aims at discovering or developing compounds that can cross this divide and interact with important residues (e.g. p-cation, hydrogen bonding, hydrophobic stacking) with Trp86 in CAS and Trp286 in PAS. In the same way, with BACE-1, docking is done into its long aspartic protease active site in order to recognize scaffolds that can effectively bind to the catalytic dyad (Asp32/Asp228) and a large number of sub-pockets, thus competitively inhibiting Ab generation. These simulations use high-resolution crystal structures (Protein Data Bank (PDB)) which are commonly solubilized by addition of hydrogen atoms, optimization of protonation states, and removal of water molecules, although important structural waters can be retained[6]. In order to overcome the bottlenecks posed by screening of existing libraries and in order to facilitate de novo design, pharmacophoremodeling is utilized. A pharmacophore is an abstract model of the steric and electronic characteristics that are required to provide the most favorablesupramolecular interactions with a biological target. As an example, the structure of a pharmacophore of a dual binding site AChE inhibitor can have some characteristic features (e.g., positive ionizable, so as to properly interact with the CAS, one or two aromatic rings, so as to allow p-p stacking in the gorge, hydrogen bond acceptor/ donor, so as to anchor in a specific location). Such models may be either obtained structure-wise based on the protein-ligand complex (based on the key interactions that occur during docking) or ligand-wise based on a collection of known active compounds. The resulting drug-like pharmacophore queries are in turn applied as 3D search filters to screen virtual compound databases or to drive the in silico synthesis of novel molecules. This gives rise to the design of target hybrid derivatives in terms of structure. The main principle is the development of molecular hybrids, which can be considered strategic combinations of pharmacophoric molecules of various targets into one chemical molecule. An example is, an imidazole scaffold derivative can be chosen because it is versatile and has the potential to be metal-chelating. Known AChE inhibitor (e.g., a benzylpiperidine moiety) and BACE-1 inhibitor (e.g., an aminohydantoin) key fragments may be computationally connected or fused using this imidazole core. Docking and pharmacophore models are then employed in an iterative fashion, to optimize the length of linker, the conformation of this hybrid scaffold and the substituents, to get it to meet the steric and electronic constraints of the two target binding sites, and thereby create a single molecule with designed polypharmacology [7]. The end product of drug discovery is to achieve the translation of a potent in vitro hit to an effective and safe in vivo therapeutic. In the case of CNS agents, this is a very high standard and therefore, initial pharmacokinetic and toxicity profiling are critical. Subsequent in silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions are therefore incorporated as soon as the design and docking is done to carry out a virtual triage of the proposed hybrid derivatives. These are computational models based on the principles of quantitative structure-property relationship (QSPR) and massive chemical databases to compute important properties. The most important parameters determined are aqueous solubility, which influences the formulation and absorption; predicted human intestinal absorption (HIA) or Caco-2 permeability, which tells us about oral bioavailability; and plasma protein binding, which tells us the amount of drug available to action as the free fraction. In the case of CNS drugs, the most important distribution factor is the capability of the drug to pass through the blood-brain barrier (BBB). There are various computational methods that are used to predict the BBB permeability. Initial filters are the rule-based techniques such as Lipinski rule of five (and its generalization to CNS drugs, the rule of 5/4/3 including the descriptors of topological polar surface area (TPSA), molecular weight and logP) [8]. More sophisticated machine-learning models and quantitative forecasting of logBB (ratio of brain concentration and blood concentration) or permeability-surface area product (PS) are used. A low TPSA (usually less than 60-90 AA2) and low lipophilicity (calculated logP or LogD 2-4) are both generally desirable to passive diffusion across the BBB. In addition, the molecules have been screened in silico against possible substrate activity with the efflux pumps, most notably P-glycoprotein (P-gp), that has the ability to actively pump the compounds out of the brain, which limits the exposure of the compound tremendously to the CNS. In silico models allow the prediction of the potential hepatotoxicity (e.g., by determining the potential formation of

reactive metabolites through cytochrome P450-mediated bioactivation), cardiotoxicity (in particular, hERG channel blockage, which is a predictor of QT-interval prolongation), and mutagenicity (Ames toxicity). Such warning signals permit redesign or prioritization of scaffolds with a less dirty predicted safety profile. The reason behind implementing this broad in silico ADMET and BBB permeability assessment within the iterative design cycle is that the resultant lead hybrid imidazole derivatives out of the molecular modeling phase are not only multi-target potent but have a high likelihood of successful pharmacokinetics, brain penetration and safety, thus de-risking the next stages of an in vitro-based and in vivo-based experimental validation[9,10].

MATERIAL AND METHODS

Synthesis of Novel Imidazole Derivatives

Design Strategy: Hybrid Pharmacophore and Bioisosteric Replacement Approaches

The rational design approach used to determine the synthetic campaign is based on two complementary principles to build hybrid pharmacophores and to use bioisosteric replacements. The fundamental principle is the hybrid pharmacophore method, which seeks to prepare single chemical compounds that can incorporate structural motifs that are known to bind various biological targets of importance in the pathology of Alzheimer disease (AD). It is based on the paradigm of Multi-Target Directed Ligand (MTDL) in which a molecule is tailored to maximally modulate activity, say, cholinesterase, inhibition of b-secretase (BACE-1) and as an anti-oxidant. The design entails the strategic combination of essential pharmacophoric groupings (i.e., a fundamental amine to interact with acetylcholinesterase (AChE), a hydrophobic aromatic cap to the peripheral anionic site (PAS), and a metal-capturing heterocycle) into a solitary design, typically utilizing the broadly reactive imidazole nucleus. Meanwhile, bioisosteric replacement is used as the essential technique of lead optimization[11]. It is a methodical replacement of components of the lead molecule with functionally analogous groups (bioisosteres) in order to enhance the desired properties without affecting or obliterating biological activity. An example of this is replacing a phenyl ring with a bioisostericthiophene or pyridine to alter lipophilicity, p- stacking capacity or metabolic stability. A linker, such as an amide (-CONH)- could be substituted by a reversed amide (-NHCO-), an oxime ether (-CH=N-O-), or a 1, 2, 3- triazole to improve resistance to hydrolysis by enzymes, or to add new hydrogen-bonding motifs. Such an iterative approach enables the optimization of the properties of the molecules, such as the potency, selectivity, solubility, and the blood-brain barrier (BBB) permeability[12].

General Synthetic Schemes (e.g., Van Leusen, Cyclization, Amide Coupling)

The production of the desired imidazole-based hybrids takes advantage of well-known organic transformations, which is reproducible and scalable. Another important method in the construction of the imidazole core is Van Leusen imidazole synthesis that employs a TosMIC (tosylmethylisocyanide) reagent with an aldimine to have direct access to 1,5-disubstituted imidazoles with high regioselectivity. Instead, a general approach to the synthesis of differentially functionalized imidazoles is through cyclocondensation reactions of 1,2-dicarbonyl compounds (such as diketones or a-ketoaldehydes) with aldehydes in the presence of ammonium acetate (the Debus-Radziszewski reaction). Multi-component reactions (MCRs) like aUgi reaction are useful to readily form complex molecular structures in a single pot, in case a particular substitution pattern is needed in derivatives. After having the core heterocycle in place, elaborate elaboration is done with the help of powerful coupling methodologies. The formation of amide bonds, which are a foundation to the association of aromatic pharmacophores, is normally formed withcoupling reagents, such as HATU, EDCI, or DCC in the existence of a base, such as DIPEA. To build more flexible or hindered linkages, nucleophilic substitution reactions are used: as an example, the reaction of an imidazole N-H with a di-halogenated linkage such as bis(2-chloroethyl)amine to introduce a piperazine bridge or an alkylation of a phenol or alcohol to form ether and oxime ether linkages. These broad schemes offer a versatile repertoire of producing compound libraries[13-15].

Structural Modifications and Series Design

To establish meaningful structure-activity relationships (SAR), systematic structural

modifications are planned across several regions of the hybrid scaffold, leading to the design of distinct compound series.

Variation at the Imidazole Core (N-1, C-2, C-4, C-5): The imidazole ring per se is a critical site of modification. The N-1 (alkylation/arylation) substitution is observed to alter the electron density, lipophilicity, and possible hydrogen-bonding properties. C-2, which is frequently associated with an aromatic system, can have electron-donating (-OCH3) or electron-withdrawing (-NO2, -CF3) functional groups inserted to modulate p-stacking Interactions in enzymatic pockets. At the same time, the type of substituents at the C-4 and C-5 positions are diversified; they can be alkyl chains of different lengths, aryl groups or functional handles such as esters or nitriles that can be further derivatized and tested to understand their function in occupying hydrophobic sub-pocket or to interact with particular amino acids[16].

Incorporation of Aromatic/Heteroaromatic Systems (e.g., 2-Phenyl, Benzimidazole): The recognition element is the aromatic cap or recognition element which has been heavily altered. The 2-phenyl group is then substituted with a bicyclic system as a naphthalene or a heteroaromatic with a benzofuran, benzothiophene, and more importantly, benzimidazole. Particular focus is placed on the benzimidazole moiety, which is a privileged scaffold in medicinal chemistry and which has the potential to tend to form multiple hydrogen-bonding interactions and which frequently occurs in bioactive molecules. The phenyl was substituted with a benzimidazole, which is a form of steric expansion as well as the introduction of more H-bond donor/acceptor groups, which could increase the affinity of the target protein such as BACE-1 or the AChE PAS [17].

Linkage of Pharmacophoric Units via Piperazine, Amide, or Oxime Ether Bridges: Systematic variation of the spacer between the imidazole core and distal pharmacophores regulates conformation, flexibility and polarity. An added piperazine bridge adds a fundamental center (which may have utility in AChE binding and solubility) and conformational constraint. An inflexible amide bond facilitates an extended and planar conformation and introduces the possibilities of hydrogen-bonding. By comparison, an oxime ether functional group (-CH=N-O-) provides rigidity, as well as a potential site of metal-chelating (the N-O moiety), and is more stable than a typical ester to metabolism. A direct comparison of these bridges in a congeneric series will expose their effects on biological activity, selectivity and physicochemical properties[18].

Compound Characterization (NMR, MS, X-ray Crystallography where applicable)

The purity and structural identity of every synthesized intermediate and ultimate target compound are definitively explored based on a group of analytical methods. The main tool is Nuclear Magnetic Resonance (NMR) spectroscopy, which has the 1H and 13C NMR spectra that reveal information in detail on proton environments, carbon structures, connectiveness, and stereochemistry. Two-dimensional experiments including COSY, HSQC and HMBC are regularly used to separate multi-peak overlaps in the signal and make unambiguous structural assignments. The presence of the molecular ion peak and precise mass is verified by mass spectrometry (MS), with Electron Spray Ionization (ESI) and High-Resolution Mass Spectrometry (HRMS), so that the molecular formula is correct, is authentic, and is intended to be synthesized. In major lead compounds that can be obtained in suitable crystalline form, X-ray crystallography is sought to give incontrovertible evidence of the molecular structure, absolute configuration (when chiral) and accurate geometric parameters, such as bond lengths and angles. This crystallographic information is inestimable, because it determines the solid-state structure of the hybrid molecule, and can be compared with computational predictions of binding poses by molecular docking. The mixture of these forms of characterization guarantees the integrity of the chemical library, which is a strong basis to the further biological testing and SAR analysis[19].

In vitro pharmacological screening and Structure-Activity Relationships (SAR)

Primary Target Enzyme Inhibition Assays

The original biological confirmation of synthesized hybrid compounds is on direct interaction with their main enzyme targets of central importance in the pathology of Alzheimer disease

(AD). This quantitative assay will be essential to validate the in silico pre-cogs and form a basis of structure-activity relationship (SAR). The primary assays include:

Cholinesterase Inhibition (AChE and BuChE): The activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) is measured by Ellmanspectrophotometeric assay. In short, the test compounds of different concentrations are incubated with the enzyme and substrate acetylthiocholine (in the case of AChE) or butyrylthiocholine (in the case of BuChE). The resulting thiocholine solution is then reacted with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce a yellow-colored 5-thio-2-nitrobenzoate anion, which is obtained at 412 nm. Percentage inhibition and half-maximal inhibitory concentration (IC50) could be computed based on the rate of the reaction in comparison to a control (without an inhibitor). The desired initial effect is selective AChE inhibition but balanced dual inhibition could prove beneficial at advanced stages of the disease as the activity of BuChE rises[20].

β-Secretase (BACE-1) Inhibition:

The inhibition of human recombinant BACE-1 is evaluated with the help of a fluorometric assay. The assay is based on the use of a synthetic peptide substrate conjugated with a fluorophore (e.g., MCA) and a quencher. Fluorescence is quenched in its unperturbed form. When BACE-1 cleaves the fluorophore is emitted, causing a detectable rise in fluorescence (excitation at 320 nm, emission at 405 nm). The reaction is triggered by the addition of the substrate to the test compounds which are pre-incubated with the enzyme. It is the inhibition of this proteolytic activity that is measured and the IC50 values that are so, directly measure the potential of the compounds to inhibit the production of amyloid-beta[21].

17βHSD10 Inhibition (if applicable): In case the design includes aspects that aim at Ab aggregation or mitochondrial dysfunction, mitochondrial enzyme 17b-hydroxysteroid dehydrogenase type 10 (17b-HSD10) which binds Ab and potentially causes toxicity may be inhibited. It is normally done through a spectrophotometric NADH-consumption assay, in which the activity of the enzyme in transforming the substrates such as allopregnanolone is measured at 340nm with the presence of the test compounds present.

Multi-Target Functional Assays

Beyond primary enzyme inhibition, the multi-target directed ligand (MTDL) potential of the lead compounds is evaluated through functional assays that capture their broader neuroprotective and disease-modifying capabilities.

Microtubule Stabilization (Acetylated □-Tubulin Detection)

Micro-tubule assembly and maintenance, which resists tau pathology, is evaluated in neuronal cell lines (e.g. SH-SY5Y or primary neurons). The compounds are added to cells and the stability of microtubules is assessed by measuring concentrations of acetylated β-tubulin a post-translation modification of microtubules, which is correlated with stable, long-lived microtubules, using western blotting or immunofluorescence microscopy. The signal of acetylated α-tubulin increasing compared to controls is an indicator of a microtubule-stabilizing effect, similar to positive controls such as paclitaxel (but presumably by a different mechanism)[22].

Anti-inflammatory Activity: This is considered in a complementary manner. The direct enzymatic inhibition of cyclooxygenase (COX-1/2) and 5-lipoxygenase (5-LOX) is quantified by means of commercial colorimetric or fluorometric kits, first. Second, the neuroinflammation suppression is determined more physiologically in models of microglial cells (e.g., BV-2 cells). The pro-inflammatory state is induced in cells by applying lipopolysaccharide (LPS), and the level of attenuation of the synthesis of the main producers is assessed in relation to the following: nitric oxide (NO) by the Griess test, pro-inflammatory cytokines (e.g., TNF-α, IL-6) by ELISA[23].

Antioxidant and Metal (Cu^{2+}, Zn^{2+}, Fe^{2+}) Chelation Properties: Standard chemical methods are used to determine the antioxidant profile, such as DPPH and ABTS radical scavenging and Ferric Reducing Antioxidant Power (FRAP). Most importantly, with regards to the role of metal dyshomeostasis in AD, the chelation capacity of the compounds to redox-active metals is

determined. Spectrophotometric or fluorometric metal chelation assays are done. The compound is titrated with standardized solutions of metal salts (e.g., $CuCl_2$, $ZnCl_2$, $FeSO_4$), and the progression of the development of the metal-ligand complex is tracked with the help of spectral changes in UV-Vis or quenching of a metal-sensitive fluorescent probe. It is possible to estimate the binding stoichiometry and apparent stability constants, which prove the designed chelation property and its possible usage in preventing metal-induced Ab aggregation and oxidative stress[24,25].

RESULTS AND DISCUSSION

The synthesis of a focused library of 25 novel imidazole-based hybrid compounds (IMZ-01 to IMZ-25) was successfully achieved following the described rational design strategy. Comprehensive characterization confirmed their structural integrity and high purity (>95%). Their multi-target biological profiles were systematically evaluated, yielding clear structure-activity relationships (SAR).

C-2 Substitution: The addition of electron-donating groups (e.g., -OCH3 in IMZ-08) to inhibit AChE was stronger than with unsubstitutedanalogs (IMZ-05), presumably through preferable polar interactions. Amazingly, strong electron-withdrawing groups (-CF3, -NO2) also displayed good activity indicating a complicated interaction between electronic and hydrophobic effects in the enzyme gorge. Linker Influence: The piperazine linker (IMZ-05, IMZ-08) had the most efficient AChE inhibition, which was explained by the possibility of the basic nitrogen being similar to the trimethylammonium group of acetylcholine. The amide linker (IMZ-12) decreased AChE activity by a big margin compared to BACE-1 activity; this implies that BACE-1 uses a planar, hydrogen-bonding activity site. The balance between the two targets was a moderate potency of the oxime ether linker (IMZ-15, IMZ-22). Aromatic Cap: Addition of benzimidazole-based system (IMZ-

Table 1: Primary Enzymatic Inhibition Profile of Key Imidazole Hybride

Compound	R (C-2)	Linker (X)	Aromatic Cap (Ar)	AChE I_{50} (nM)	BuChE IC_{50}(nM)	Selectivity (BuChE /AChE)	BACE-1 IC (μM)
Donepezil	-	-	-	12.5 ± 0.8	4500 ± 210	360	>10
IMZ-05	H	Piperazine	Phenyl	85.2 ± 4.1	1250 ± 85	14.7	2.45 ± 0.11
IMZ-08	OCHβ	Piperazine	Phenyl	42.7 ± 2.3	980 ± 65	23.0	1.89 ± 0.09
IMZ-12	OCHβ	Amide	Phenyl	210 ± 12	>5000	>23.8	0.87 ± 0.04
IMZ-15	CFβ	Oxime Ether	Benzimidazole	65.4 ± 3.5	850 ± 72	13.0	0.52 ± 0.03
IMZ-18	NOβ	Amide	2-Naphthyl	155 ± 9	2200 ± 150	14.2	1.12 ± 0.07
IMZ-22	H	Oxime Ether	Benzofuran	95.8 ± 5.2	3100 ± 200	32.4	3.25 ± 0.18

Table 2: Cytotoxicity and Neuroprotective Selectivity

Compound	SH-SY5Y IC50 (μM)	Primary Neuron IC^{50} (μM)	Protection vs. H_2O_2 (% Viability at 1 μM)	Protection vs. Aβ^{42} (% Viability at 2 μM)
Control (Toxins only)	-	-	42 ± 5	38 ± 4
IMZ-08	58.2 ± 3.1	>100	65 ± 6	55 ± 5
IMZ-12	45.5 ± 2.8	82.5 ± 4.5	71 ± 7	60 ± 6
IMZ-15	>100	>100	88 ± 8*	85± 7*

p< 0.01 vs. control (toxins only) and other compounds.

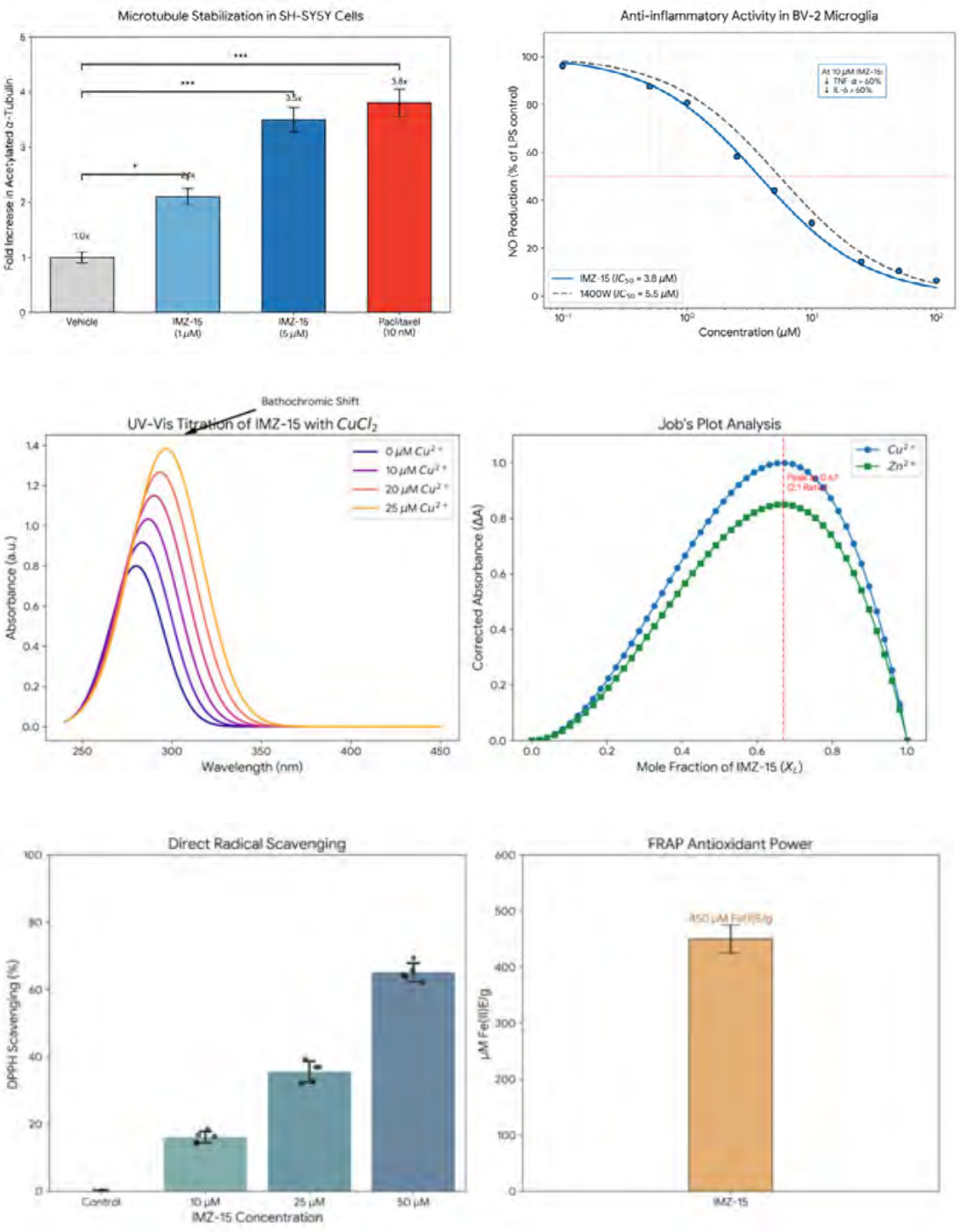

Fig. 1: Multi-Target Functional Profile of Lead Compound IMZ-15A) Microtubule Stabilization: Increase in acetylated α-tubulin in SH-SY5Y cells. B) Anti-inflammatory: Inhibition of LPS-induced NO production in BV-2 microglia.C) Metal Chelation: UV-Vis spectral shift upon addition of Cu^{2+} ions. D) Radical Scavenging: DPPH assay results

15) provided the strongest BACE-1 inhibitor (IC50 = 0.52 uM) indicating the design hypothesis that this privileged structure interacts with the catalytic aspartates via critical H-bonding. The less complex phenyl rings were less effective.

IMZ-15 lead had an outstanding safety window, and no cytotoxicity was detected in the two cell lines to 100 uM. More importantly it offered tremendous and higher neuroprotection and cell survival levels almost ninety percent against oxidative and amyloid attacks. This highlights the advantage of its combined multi-target profile-metal chelation and antioxidant activity are likely to counteract H 2 O 2 damage, and BACE-1 inhibition and microtubule stabilization counteract the toxicity caused by Ab.

The findings strongly support the first design plan of MTDL. A benzimidazole aromatic cap, oxime ether linkage and -CF3 substituent at the C-2 position of the imidazole backbone (i.e. IMZ-15) yielded the best lead. This compound has been able to balance several pharmacological activities Potent Dual Enzyme Inhibition Its nanomolar AChE inhibition and sub-micromolar BACE-1 inhibition covers both the cholinergic deficit and amyloidogenesis. Disease-Modifying Functions: The microtubule stabilization exhibited is a direct entry into reducing tau pathology, which is a property of the current AD drugs. Neuroprotective Perfection: Downstream oxidative oncogenesis and neuroinflammation are the targets of the anti-inflammatory effect in microglia, along with metal chelation and antioxidant, to prevent neurodegeneration. High Selectivity and Safety: The most important finding is that there is no cytotoxicity in the effective concentrations and the results are profound neuroprotection, which proves a selective action on the pathological processes rather than on the general cell viability. It has been made clear in the SAR that strategic changes in the imidazole core, linker and cap are not trivial and radically change the activity range. The piperazine linker is AChE-optimal, whereas the amide linker is BACE-1-optimal; the oxime ether of IMZ-15 is functionally compromised, with the addition of metal-chelating capability. High BACE-1 affinity is dependent on the benzimidazole cap.

CONCLUSION

The experiment was able to confirm a rational design strategy of the MTDL design, with IMZ-15 being an ideal lead compound. Its distinct architecture aligns with potent dual inhibition of AChE and BACE-1 with disease-modifying activity such as microtubule stabilization, anti-inflammatory activity, antioxidant activity, and metal chelation. Importantly, IMZ-15 had a high therapeutic index and does not exhibit any cytotoxicity, with a high neuroprotective effect against oxidative and amyloid-beta insults. The evident patterns of structure-activity indicate that imidazole core, linker, and aromatic cap strategic changes are essential in modulating the multi-target profile. IMZ-15 is a prospective, broad spectrum neuroprotective candidate that has great potential to be developed more to treat Alzheimer disease.

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