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BIGUANIDE DERIVATIVE:STRUCTURE, AChE INHIBITION, DOCKING

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Abstract : Alzheimer's disease (AD) burdens over 55 million globally, demanding personalized therapies amid its heterogeneity. Biomarkers within ATN framework -amyloid (A), tau (T), neurodegeneration (N)-precisely detect pathology via CSF, PET, and plasma p-tau217, patient stratification for anti-amyloid drugs like lecanemab. Intriguingly, metal-biguanide coordination compounds, with their established antimicrobial properties, emerges as novel AD candidates; biguanides like metformin reduce Aβ aggregation and tau hyperphosphorylation, while metal complexes enhance brain penetration and neuroprotection. Integrating biguanide-based biomarkers could revolutionize therapy guidance, tailoring interventions to individual pathology profiles for optimal efficacy.[123]

IndexTerms **-Alzheimer's disease, ATN framework, p-tau217 biomarker, metal-biguanide compounds, personalized therapy.**

INTRODUCTION

Alzheimer's disease (AD) represents one of the most pressing neurodegenerative challenges, affecting over 55 million people worldwide and projected to triple by 2050 due to aging demographics. This progressive disorder, characterized by amyloid -beta (Aβ) plaques, tau tangles, and neuronal loss, exhibit profound heterogeneity in onset, progression and treatment response, rendering one-size-fits all therapies largely ineffective. Biomarkers have emerged as pivotal tools to navigate this complexity, enabling precise diagnosis, patient stratification, and personalized therapy in line with ATN framework-amyloid (A), tau (T) and neurodegeneration (N).

Core ATN biomarkers include cerebrospinal fluid (CSF) Aβ42 and amyloid PET for A, phosphorylated tau (p-tau) species like p-tau217 via plasma assays for T and structural MRI atrophy or CSF total tau for N, achieving diagnostic accuracies exceeding 90%. These markers facilitate early detection years before symptoms, enriching clinical trials for targeted interventions such as anti-amyloid monoclonal antibodies (e.g., lecanemab, donanemab), which slow decline in A-positive early-stage patients while mitigating risks like amyloid-related imaging abnormalities (ARIA) in APOE4 carriers.

By guiding therapy selection and monitoring- e.g., tracking p-tracking p-tau217 reductions post treatment-biomarkers herald a shift towards precision medicine, optimizing outcomes amid AD's multifactorial pathology. This introduction explores their role in revolutionizing AD management.

NEED OF THE STUDY.

Alzheimer's disease (AD) represents a pressing global health challenge, characterized by progressive cognitive decline, memory loss, and neuropsychiatric disorders due to neurotransmitter imbalances, particularly acetylcholine deficiency in the brain. Current cholinesterase inhibitors like donepezil, rivastigmine, and galantamine elevate acetylcholine levels by inhibiting acetylcholinesterase (AChE), yet they often cause side effects such as gastrointestinal issues and hepatoxicity, underscoring the need for safer, more effective alternatives.

Biguanides, historically used in antidiabetic, antimalarial, and antiseptic applications, exhibit diverse pharmacological potential, including anti-Alzheimer's activity, but their AChE inhibitory mechanisms remain underexplored. This study addresses this gap by synthesizing a biguanide derivative (L1.HCl) from 4-ethylaniline and dicyandiamide, alongside its 1,3,5- triazine cyclic analogue (L2.2HClO$_4$), followed by structural characterization via X-ray diffraction, spectroscopic analysis, and evaluation of AChE inhibition. Docking simulations and ADMET predictions further validate L1. HCl's promising binding affinity, drug-likeness per Lipinski's rules, and favorable pharmacokinetic profile, positioning these compounds as potential multipotent leads for AD therapy with reduced toxicity. By elucidating structure-activity relationships, this work advances biguanide-based cholinergic agents amid the rising AD burden.

RESEARCH METHODOLOGY

The methodology encompasses compound synthesis, structural characterization, biological assays, computational docking, and ADMET predictions for biguanide derivatives targeting acetylcholinesterase (AChE) inhibition. All experiments followed standard organic synthesis and analytical protocols, with commercial reagents used without purification.

3.1 Data and Sources of Data

The research generates primary data on biguanide derivatives L1.HCl and L2.2HClO$_4$, including synthesis yields (L1.HCl: 65%;L2.HCl: 72%), spectroscopic (FT-IR, 1H/13C NMR), elemental analysis, and X-ray structures (monoclinic P21/n for L1.HCl; triclinic P-1 for L2.2HCLO$_4$; bond length 1.30-1.36A^o). AChE inhibition data :L1.HCl (14.76%, 34.65%, 52.10% at 1-6 mM; IC50 5.34 mM), via Ellman's method on electric eel AChE(Sigma-Aldrich) using UV-1800. Docking (DS 2018, AChE PDB:4EY6) yields binding energies (L1.HCl:-16.96kcal/mol); ADMET via DS (Lipinski compliant for L1.HCl).

3.2 Theoretical framework

Alzheimer's disease stems from acetylcholine deficiency due to excessive acetylcholinesterase (AChE), justifying cholinesterase inhibitors as therapy. Biguanide exhibit versatile bioactivity; this study theoretically links their C-N-C-N-C conjugated systems to AChE inhibition via hydrogen bonding (Ser125, Tyr133), electrostatic (His447), and hydrophobic interactions (Trp86, Tyr337) in AChE 's active site (PDB:4EY6), validated by CDOCKER docking and Lipinski-compliant ADMET profiles

3.3 Materials and General Methods

Starting materials (4-ethylaniline, dicyandiamide, HCl, HClO$_4$, acetone) and solvents were procured from commercial sources and applied as received. FT-IR spectra recorded on PerkinElmer Paragon 1000 PC spectrometer using KBR pellets. NMR spectra (1H and 13C) were acquired in DMSO-d6 on unspecified instruments, with chemical shifts reported in ppm relative to TMS. Elemental analysis were conducted via standard CHN combustion methods. AChE enzyme (E.C.3.1.1.7) from electric eel was sourced from Sigma-Aldrich.

3.4 X-ray Crystallography

Single crystals of L1.HCl and L2.2HClO$_4$ were grown from ethanol solutions. Data collection and cell refinement used a Bruker Apex II CCD diffractometer with Mo-Kα radiation (λ= 0.71073 A^O) at ambient temperature. Bruker SAINT software processed raw data, including indexing, integration, and scaling. Structures were solved by direct methods with SHELX-2014 and refined anisotropically on F^2 using all reflections via full matrix least-squares. Hydrogen atoms were placed geometrically and refined isotropically; no absorption corrections were detailed beyond standard procedures.

3.4.1 Crystal Data and Refinement Statistics

Parameter	L1.HCl	L2.2HClO$_4$
Crystal System	Monoclinic	Triclinic
Space Group	P21/n	P-1
Unit Cell Parameters		
-a (A^o)	9.4058	7.6711
-b (A^o)	15.4992	8.2745
-c (A^o)	17.3333	15.7375
-α (^{o})	90	86.2215
-β (^{o})	102.3015	86.5446
-γ (^{o})	90	82.3356
Unit Cell Volume (A^{o3})	2468.96	986.52
Refinement Statistics		
-R1 (I> 2σ(I))	0.0668	0.0953
-wR2 (I> 2σ(I))	0.1353	0.2170
CCDC Deposition Number	1892331	1892332

3.5 Synthesis of L1.HCl and L1.2HClO$_4$

L1.HCl was synthesized by heating 4-ethylaniline (4.12g, 34 mmol) and dicyandiamide (2.85 g, 34mmol) in 3M HCl (11.22 ml) at 90^o C for 18hr under reflux. The reaction mixture cooled to room temperature, stood 1-3 hr, and the precipitate was vaccum-filtered, washed with cooled water, yielding white L1.HCl (5.33 gm, 65%). For L2.2HClO$_4$,HCl was replaced with 3M HClO$_4$, affording light brown product (5.33gm, 65%).

3.5.1 Characterization Data for L1.HCl($C_{10}H_{16}N_5Cl$, MW 241.72)

Technique	**Details**
Elemental Analysis	Calc: C 49.69%, H 6.67%, N 28.97% Found: C 49.32%, H 6.45%, N 28.33%
FT-IR (KBr, cm^{-1})	3301-3148($\nu N-H$)2971 ($\nu C-H$ *aliphatic*)2193/2155 ($\nu C\equiv N$)*absent* ($\nu N-H$)616/614
^{1}H NMR (DMSO- d6, ppm)	1.18(3H,t,Ph-CH$_2$-CH$_3$)2.56(2H,q,Ph-CH$_2$-CH$_3$)7.04/7.14 (4H,d, Ar-H) 7.26 (6H, s, NH$_2$) 9.62

	(1H,s,NH_2)
^{13}C NMR (DMSO-d6, ppm)	15.62 (Ph- CH_2-CH_3) 28.42 (Ph- CH_2) 122.33/127.43/131.68/144.45 (Ar-C)158.16/159.13 (N=C-N)

3.5.2 Characterization Data for L2.2HClO$_4$($C_{13}H_{21}N_5Cl_2O_8$, MW 446.24)

Technique	Details
Elemental Analysis	Calc: C 34.99%, H 4.74%, N 15.67% Found: C 34.65%, H 7.52%, N 15.10%
FT-IR (KBr, cm^{-1})	3338-3272($\nu N-H$)2968 ($\nu C-H$ *aliphatic*)1659/1637 ($\nu C\equiv N$)619/522
^{1}H NMR (DMSO- d6, ppm)	1.22(3H,t,Ph-CH_2-CH_3)1.29(6H,q,Ph-CH_2-CH_3)6.19/7.55 (4H,d, Ar-H) 9.23 (1H, s, NH)
^{13}C NMR (DMSO-d6, ppm)	15.33/27.51(ethyl) 28.16/69.95 (-C(CH_3)$_2$) 129.79/130.15/132.88/145.67 (Ar-C)158.34/157.85 (N=C-N)

3.6 AChE Inhibitory Activity Assay

AChE inhibition followed Ellman's method. Enzyme activity was monitored at 412 nm (Shimadzu UV-1800 spectrophotometer, 1Mm cuvettes). Compounds L1.HCl and L2.2HClO$_4$ dissolved in 2% methanol at 1,3,6 Mm concentrations. Assay mixture: 100μL AChE (0.5 U/mL), 100μL DTNB (0.01M), 100μL substrate (acetylthiocholine iodide, 15mM), and 700μL buffer (Ph 8.0). % Inhibition= [Abs control -Abs sample)/Abs control]×100; IC50 interpolated from dose-response curves.

3.7 Molecular Docking Studies

Performed in Discovery Studio (DS) 2018 AChE structure prepared via "Prepare Protein" protocol: cleaned, protons added, bonds optimized. Ligands (L1.HCL, L2HCLO$_4$) prepared with "Prepare Ligands" (CHARMm force field, ABNR minimization to RMSD gradient 0.05 kcal/mol A^{o2}) . Active site defined from literature and DS tools. Docking via CDOCKER :10 poses/ligand, scored by CDOCKER energy, interaction energy, binding energy (kcal/mol), RMSD (A^o). Galantamine docked as reference. Interactions analyzed :H-bonds (Ser125, Tyr133), electrostatic (His447,Trp86), hydrophobic (Tyr337, Tyr341, Trp286).

3.8 In Silico ADMET Analysis

DS 2018 assessed Lipinski/Veber Rules Thresholds

Parameter	Lipinski Rule	Veber Rule
MW(Da)	≤500	≤500
Log P	≤ 5	≤ 5
HBA (H-bond acceptors)	≤10	≤12
HBD (H-bond donors)	≤ 5	≤ 12
NRB (Rotatable bonds)	≤10	-
MPSA (A^{o2})	≤ 140	≤140

3.8.1 ADMET Computed descriptors

Descriptor	Scale/Range	Notes
AlogP98	-	Lipophilicity (octanol-water)
PSA (Polar Surface Area)	A^{o2}	-
HIA(Human Intestinal Absorption)	0-1 (0=good)	-
Solubility	0-4(0-2 optimal/good)	Aqueous solubility level
BBB	0-4(0-2 low/medium)	Penetration level
CYP2D6	Boolean (False=no)	Cytochrome P450 2D6
Hepatotoxicity	Boolean (False=no)	Toxicity prediction
PPB (Plasma Protein Binding)	%(<90 weakly bound)	Binding to carrier proteins

Visualization:95/99% confidence ellipses plotted for HIA vs BBB models.
This comprehensive methodology integrates wet-lab synthesis/characterization with in vitro bioassays and in silico predictions, ensuring robust validation of compounds AChE potential for Alzheimer's therapy.

RESULTS AND DISCUSSION

Category	L1HCl	L2.2HCl0$_4$
AChE Inhibition (%)(1/3/6 mM)	14.76/34.65/52.10	10.36/28.96/55.94
IC50(μM)	5.54	5.34
Docking Binding Energy(kcal/mol)	-16.96	-12.89
CDOCKER Energy(kcal/mol)	-40.27	-37.37
Lipinski Compliance	Yes (MW 242,LogP 1.21,HBA 5,HBD 7)	No (HBA 13, HBD 10)
ADMET Highlights	HIA good, Sol 4 (optimal), BBB low, non-toxic, PPB weak	HIA good, Sol 4, BBB low, hepatotoxic

The synthesized biguanide L1HCl and cyclic analogue L2.2HCl0$_4$ were fully characterized, confirming protonation at terminal imino groups (L1HCl) and triazine nitrogens (L2.2HCl0$_4$), with delocalised Π-elelctrons evident in C-N bonds (1.32-1.35A^o) and hydrogen-bonded networks. Both exhibited moderate AChE inhibition, with IC50 values of 5.54 μM L1HCl) and 5.34 μM (L2.2HCl0$_4$) at 1-6 mM concentrations, outperforming none but approaching galantamine (4.48 μM), though weaker than donepezil (0.054μM). Lower potency attributed to suboptimal enzyme-ligand conformation.

Docking in AChE (PDB:4EY6) revealed L1HCl' s superior binding (-16.96kcal/mol), with H-bonds (Ser125, Tyr133), electrostatics (His447, Trp86), and hydrophobics (Tyr337/341, Trp286), closely mimicking galantamine's pose. L2.2HCl0$_4$ showed weaker interactions (-12.89 kcal/mol) due to mismatched orientation. CDOCKER energies corroborated experimental IC50 trends.
ADMET profiling (DS 2018) confirmed L1HCl's superior binding (Lipinski: MW242 Da, LogP 1.21, MPSA 142 A^{o2}), good HIA, optimal solubility, low BBB penetration, no CYP2D6/ hepatotoxicity, and weak PPB- ideal for AChE inhibitors despite modest brain permeability. L2.2HCl0$_4$ violated Lipinski/Veber (HBA 13,HBD 10, MPSA 232 A^{o2}) and predicted hepatotoxic, limiting viability. These findings position L1HCl as a promising AChE inhibitor lead, leveraging biguanide's multipotent bioactivity for Alzheimer's, warranting further optimization.

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