Research Article

New Approach for TargetingSmall Molecule Candidates for Intrinsically Disordered Proteins

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Intrinsically disordered proteins (IDPs), like the Alzheimer's associated tau protein, pose challenges for conventional drug discovery. This study applied the Informational Spectrum Method for Small Molecules (ISM-SM), a computational technique utilising electron-ion interaction potentials (EIIP), to identify potential tau modulators. Characteristic interaction frequencies derived from known ligands and conserved mammalian tau sequences were used to screen DrugBank and the COCONUT natural product database. The screening identified approved drugs previously reported to indirectly influence tau pathology or Alzheimer's disease pathways, alongside natural products like Bryostatin-14, known to modulate kinases involved in tau phosphorylation. These findings suggest ISM-SM can serve as an in silico tool to identify candidate small molecules, including repurposed drugs and natural products, with potential relevance to tau function and pathology, complementing other IDP drug discovery strategies.

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1. Introduction

Intrinsically disordered proteins (IDPs) have essential roles in a variety of biological processes and have been associated with numerous diseases, including neurodegenerative disorders and viral infections[1][2]. In contrast to structured proteins, which contain stable binding pockets, IDPs exist as dynamic conformational ensembles and represent particularly challenging targets in small-molecule drug discovery. Several small molecules have been identified as IDP ligands, usually by binding to transient interaction sites or as modulators of their dynamic conformational states. Notable examples include

epigallocatechin gallate (EGCG) for α-synuclein[3], Phenothiazine for tau[4], and different compounds targeting viral nucleocapsid proteins[5]. IDPs' inherent flexibility leads to specialised experimental and computational methods to identify their ligands.

Conventional structure-based drug development methods are not as successful with IDPs because the binding sites are poorly defined. Similarly, high-throughput virtual screening methods struggle with IDP dynamic and heterogeneous nature. They may call for alternate techniques like ensemble docking[6][7][8][9][10][11], molecular dynamics (MD) simulations[12][13][14][15][16][17][18][19][20], and machine learning models trained using IDP-ligand interactions[16][21][22][23][24][25]. Experimental methods, including nuclear magnetic resonance (NMR) spectroscopy, surface plasmon resonance (SPR), and fluorescence-based assays, can also help demonstrate weak, transient interactions[26]. Therefore, computational and biophysical methods are crucial for discovering and optimising small-molecule ligands.

The standard new drug development process typically involves hit identification, lead optimisation, preclinical testing, and clinical trials. The cost of bringing a drug to market follows the industry standard, averaging $1.39-2.87 billion over 10-15 years, with high attrition rates due to the complexity of validating functional effects and ensuring specificity.[27] Due to the additional perplexity of IDPs-ligand interactions, this process is more challenging, and the introduction of various *in silico* methods is required.

The ISM-SM (Informational Spectrum Method for Small Molecules) method offers a distinct approach compared to traditional High-Throughput Screening (HTPS) methods for finding small-molecule candidates for disordered proteins, particularly by analysing long-range interaction potentials rather than relying solely on structure[28]. ISM-SM can put molecular structures into a frequency spectrum, enabling it to identify the compatible interaction frequencies for small molecules and target proteins, predicting biological activity. Our previous ISM-SM studies have successfully identified biologically active ligands for specific protein binding sites[29][30]. ISM-SM has also been utilised to discover binders towards proteins associated with emerging viral threats, such as SARS-Cov-2. An example is the determination of the interaction frequencies between small molecules that have been shown to have benefit against viral proteins, predicting drugs that could be repurposed for COVID-19, and significantly accelerating the drug discovery process in response to a pressing public health emergency. [31][32][33]. This work explores the application of ISM-SM for identifying potential therapeutic candidates against

selected disease targets. We will here focus on human tau protein candidates, one of the two hallmark proteins of Alzheimer's disease (AD).[34]

2. Materials and methods

2.1. Databases

The sequence of Human microtubule-associated protein tau (P10636) was taken from the UniProt database (www.uniprot.org)[35]. Tau proteins from other mammals were also downloaded:

- O02828 Capra hircus
- P10637 Mus musculus
- P19332 Rattus norvegicus
- P29172 Bos taurus
- P57786 Macaca mulatta
- Q5S6V2 Pongo pygmaeus
- Q5YCV9 Hylobates lar
- Q5YCW0 Gorilla gorilla gorilla
- Q5YCW1 Pan troglodytes
- Q6TS35 Spermophilus citellus
- Q9MYX8 Papio hamadryas

For screening of drugs for repurposing to select candidates for Microtubule-associated protein tau, 2627 approved small molecule drugs from DrugBank[36] (http://www.drugbank.ca) were screened. The criteria for candidate selection were five reported tau protein binding drugs: DB00637 Astemizole, DB01248 Docetaxel, DB14914 Flortaucipir F-18, DB00448 Lansoprazole, and DB01229 Paclitaxel.

The Coconut database[37] is a freely available collection of over six hundred thousand natural products, some of which may also be commercially available (https://coconut.naturalproducts.net/). The Coconut database was downloaded, and compounds were converted to SMILES notation.

2.2. ISM-SM method

In this work, we analyse the tau protein and its small molecule ligands using the Informational Spectrum Method for Small Molecules (ISM-SM), the extension of the Informational Spectrum Method (ISM).

This bioinformatics approach encompasses three basic steps:

i. the representation of the protein's primary structure as a numerical sequence by assigning to each amino acid the corresponding value of the electron-ion interaction potential (EIIP),
ii. the representation of a small molecule's structure in the SMILES notation as a numerical sequence by assigning to each atomic group the corresponding value of the electron-ion interaction potential (EIIP),
iii. the transformation of the obtained numerical sequences into the informational spectrum (IS), and (iv) the calculation of the cross-spectrum (CS) between interacting protein and small molecules.

These values correspond to the electron–ion interaction potential (EIIP), determining the electronic properties of amino acids/nucleotides, which are essential for their intermolecular interactions. The EIIP descriptors are easily calculated using the following formulas:

The EIIP is the physical parameter determining organic molecules' long-range interactions (distances 5 – 1000 Å[38][39]). The following equation defines this molecular descriptor[40][41]:

$$W = 0.25\frac{Z^* \times \sin(1.04\pi Z^*)}{2\pi}, \tag{1}$$

Where Z* is the average quasi-valence number (AQVN):

$$Z^* = \frac{1}{N}\sum_{i=1}^{m} n_i Z_i, \tag{2}$$

(2) N is the total number of atoms, n_i is the number of atoms of the i-th component, Z_i is the valence number of the atomic element in the molecule, and m is the number of components. The EIIP values calculated according to Eq. (1) are given in Rybergs (Ry).

The numerical sequence, representing the primary structure of a protein, is transformed into the informational spectrum by the discrete Fourier transformation:

$$X(n) = \sum_{m=1}^{N} x(m)e^{-i2\pi(m-1)/N}, n = 1, 2, \ldots, N/2 \tag{3}$$

(3) X(m) represents the m-th element of a given numerical series, with N being the total number of points in that series, and X(n) is the coefficient of the discrete Fourier transformation. This transforms the information contained in the sequence of amino acids into a series of frequencies and their corresponding amplitudes. The informational spectrum (IS) frequencies reflect the distribution of structural motifs with specific physicochemical properties, which are crucial in defining a protein's

biological function. The informational spectrum method (ISM) can identify frequency/code pairs specific to their shared biological characteristics or related interactions when comparing proteins with similar biological or biochemical functions. The common spectrum (CS) highlights these shared informational features of the protein sequences:

$$C(j) = \prod_{i=1}^{N} S(i,j) \tag{4}$$

(4) C(j) refers to the j-th element of the common spectrum (CS), while S(i, j) is the j-th element of the i-th informational spectrum (IS). The standard information encoded in the primary structures of the proteins being analysed is captured by the frequencies in the CS. These frequencies correspond to the proteins' typical biological function or shared interactors examined through the ISM analysis. In the CS, the amplitude indicates the strength of the interaction, and the signal-to-noise (S/N) ratio reflects the specificity of the interaction between the two proteins or a protein and a small molecule.

From common frequencies in CS, one can determine whether a protein interacts with other proteins (protein-protein interactions, PPI) or small molecules and identify the corresponding binding region in the protein.

Slide window analysis identified the protein domains responsible for intermolecular recognition and targeting.

2.3. Drug Score Calculation

Drug Score (dS) values were calculated in DataWarrior.[42] The following descriptors required were calculated (s): druglikeness, logP, logS, Molecular Weight (MW), and four types of drug toxicity (t): primary irritation, mutagenic effects, reproductive effects, and tumorigenic effects. The druglikeness in the DataWarrior is partially based on topological descriptors, fingerprints of MDL structure keys or other properties as cLogP and molecular weights, including a list of about 5300 distinct substructure fragments with associated druglikeness scores. The druglikeness is calculated with the following equation, summing up score values of those fragments that are present in the molecule under investigation:

$$d = \frac{\sum v_i}{\sqrt{n}} \tag{5}$$

Drug score was calculated according to the following formulas:

$$dS = \prod \left(\frac{1}{2} + \frac{1}{2} s_i \right) \cdot \prod t_i, \tag{6}$$

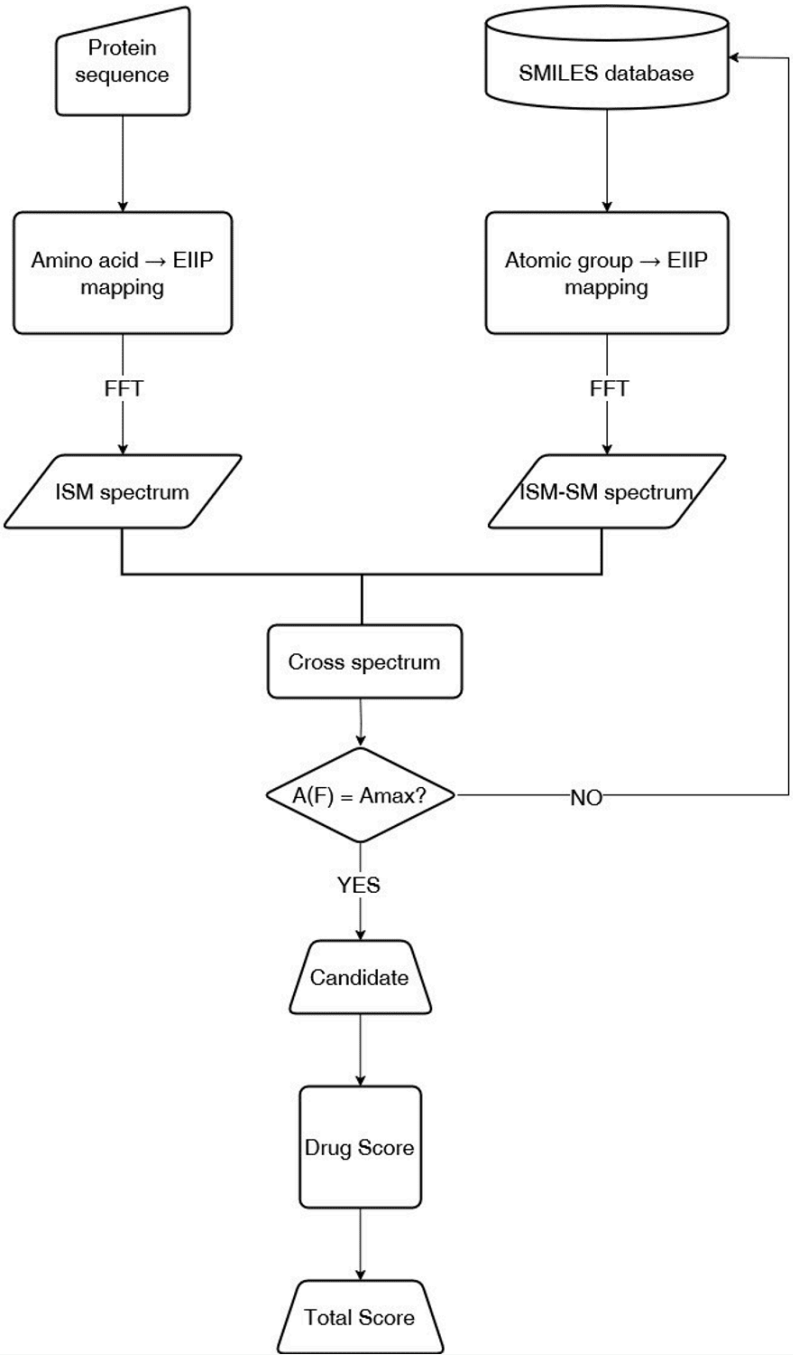
$$s_i = \frac{1}{1 + e^{ap+b}} \tag{7}$$

Where p corresponds to logP, logS, MW and Druglikeness, parameters a and b correspond to values {1, -5}, {1, 5}, {0.012, 6}, {1, 0}, respectively. The t_i values are 1.0, 0.8 and 0.6 for no risk, low and high risk, respectively. A detailed reference on the calculation of molecular properties can be found in the DataWarrior manual at https://openmolecules.org/properties/properties.html.

The Total Score had to incorporate long-range interaction properties, measured by ISM-SM signal-to-noise ratio (S/N) and Drug Score. As both parameters favour the higher values, the Total Score is therefore calculated as their product, i.e.

$$\text{Total Score } = dS \times S/N \tag{8}$$

The full methodology workflow is presented in Scheme 1.



Scheme 1. Methodology workflow. A(F) – value of the ISM amplitude at the frequency F. Amax – maximum amplitude value among all frequencies.

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3. Results and discussion

3.1. Drugbank candidates

Five reported drugs from the Uniprot database targeting Microtubule-associated protein tau protein (P10636) were extracted with their structures in SMILES format (Figure 1). The structures were converted into explicit hydrogen format, and their CS with the tau protein were calculated, along with the corresponding interaction domains (Table 1). Five frequencies, F(0.080), F(0.167), F(0.194), F(0.342) and F(0.435), were identified (Figure 2). The additional frequency, from CS including all five drugs and tau protein, was found at F(0.333) (Figure 3). Interestingly, the same frequency was obtained from the CS spectrum of all mammal tau proteins (Figure 4). This suggests an evolutionarily conserved region in the tau protein.

Regarding the binding domains of tau protein, Astemizole, Flortaucipir F-18 and Lansoprazole bind to R3-R4 regions[43][44][45], while Docetaxel and Paclitaxel don't bind directly to tau, but β-tubulin. The calculated domains corresponding to CS frequencies agree with the literature, even indirectly, due to partial spanning of the Microtubule-Binding (MTBD) region (244-368)[46] (Figure 5). The region corresponding to F(0.333) corresponds to the residues 494-750. This C-terminal domain is, however, not directly responsible for microtubule binding, but rather may modulate or influence this interaction indirectly under certain conditions, such as phosphorylation or aggregation.[47]

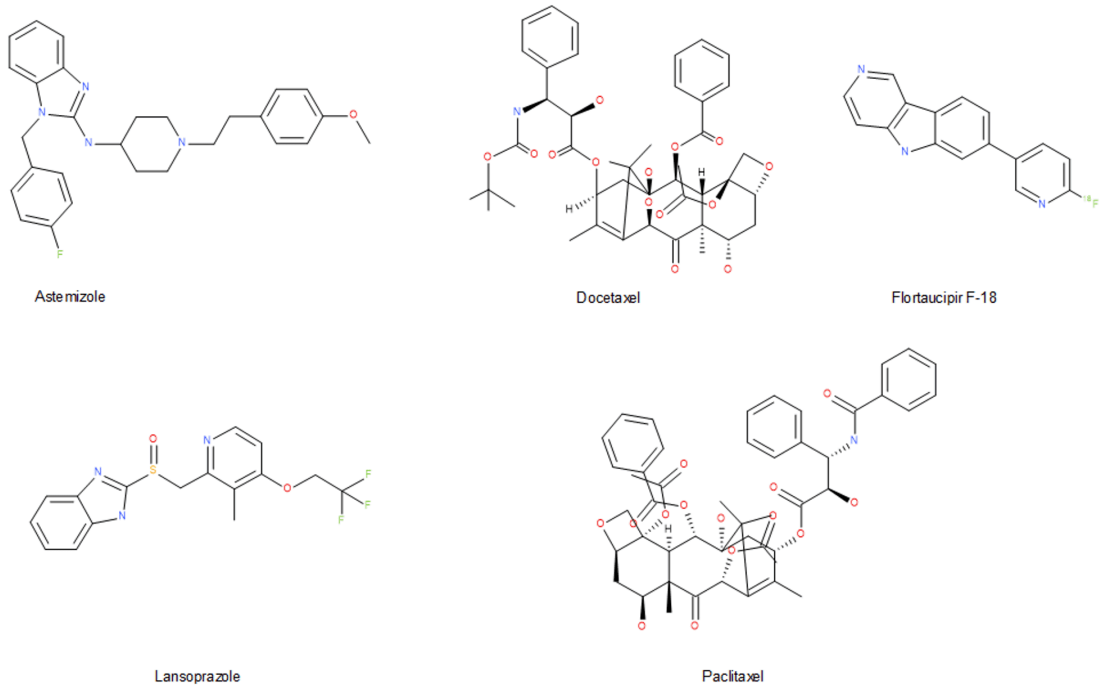

Figure 1. Structures of Drugbank compounds directly binding to the tau protein

Drugbank compound	Name	CS with tau frequencies	Amplitude	S/N	Corresponding domain in the tau protein	Literature binding region
DB00637	Astemizole	0.342	0.9958	17.353	269-525	386-391
DB01248	Docetaxel	0.167	1.7847	20.023	62-318	β-tubulin
DB14914	Flortaucipir F-18	0.080	0.10816	9.0225	427-683	R3-R4 386-391
DB00448	Lansoprazole	0.435	0.65514	12.299	305-561	R3-R4 386-391
DB01229	Paclitaxel	0.194	1.1917	14.089	23-279	β-tubulin

Table 1. Tau-interacting compounds from the Drugbank, with corresponding frequencies from CS spectra with the tau protein.

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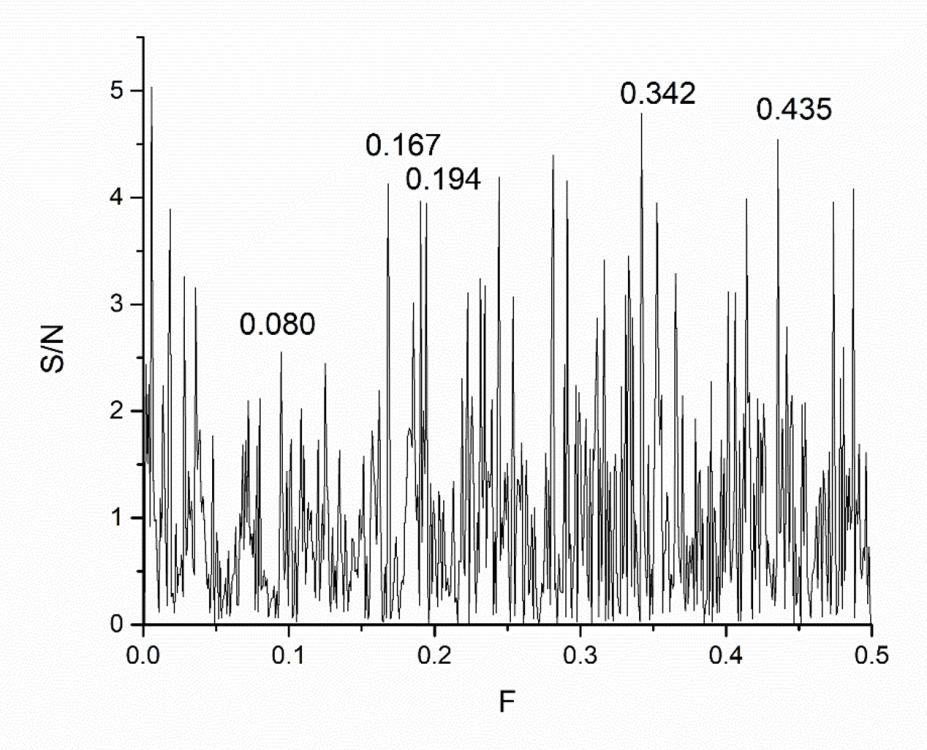

Figure 2. ISM spectrum of the tau protein

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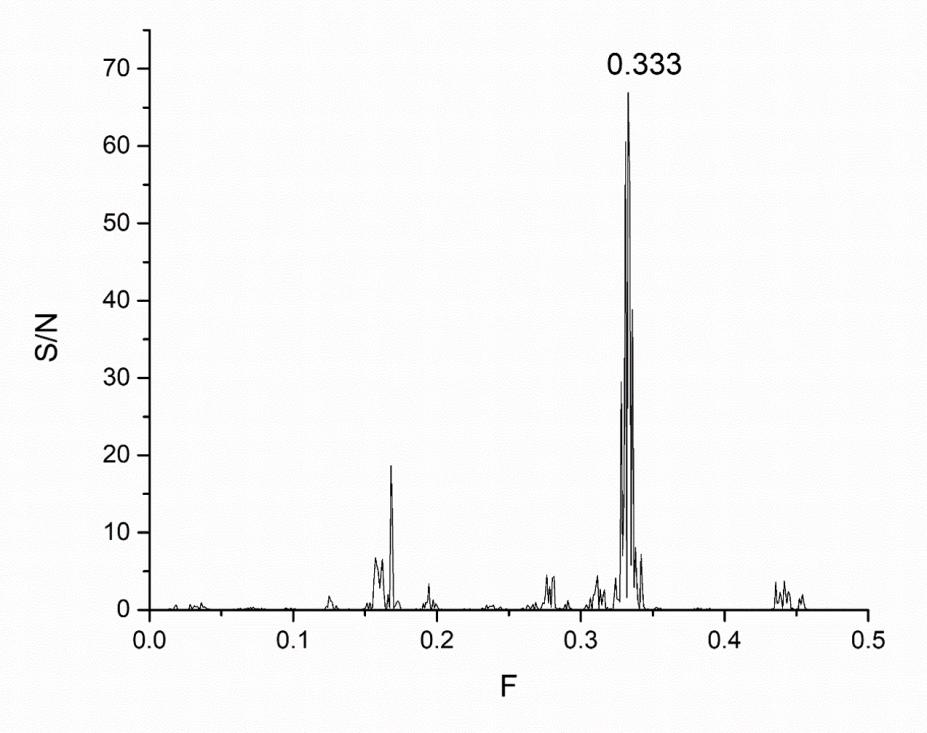

Figure 3. Cross-spectrum of all five tau protein targeting drugs from Drugbank

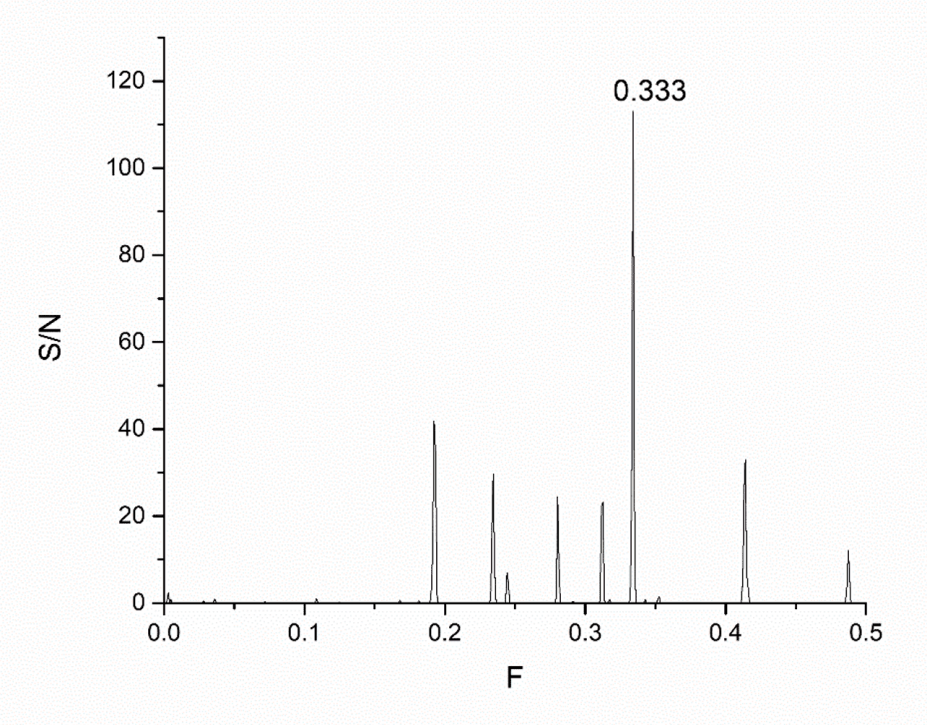

Figure 4. Cross-spectrum of all mammal tau proteins

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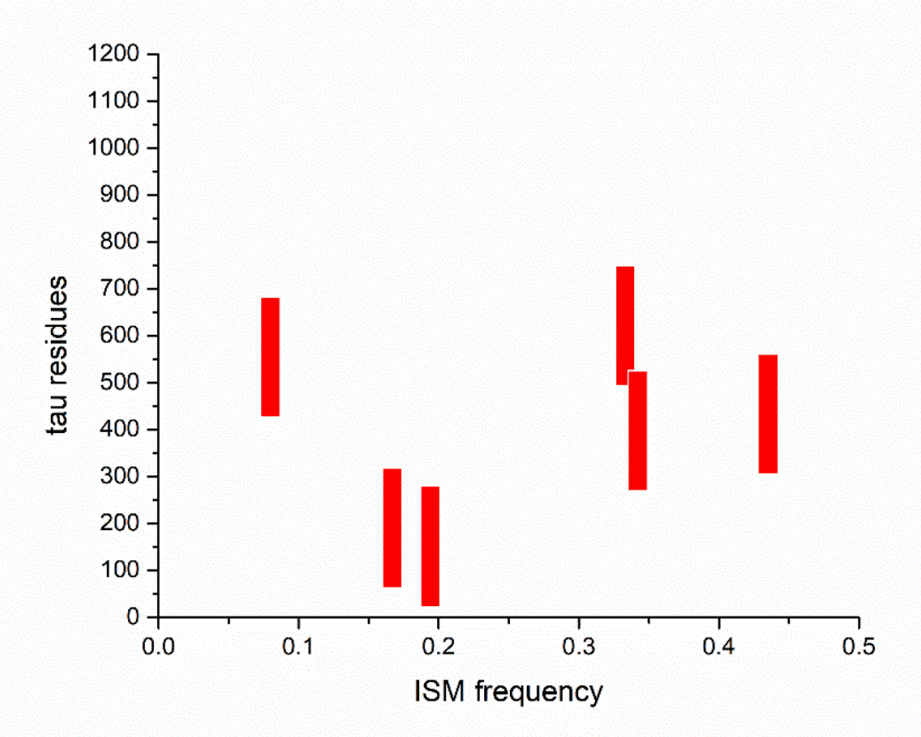

Figure 5. The domains in the tau protein corresponding to CS frequencies with the ligands

The 2627 approved Drugbank candidates' structures were subjected to the exact format conversion as literature compounds and were further CS scanned at all six frequencies. Of the candidates obtained (Supplementary material), 19 were already reported to indirectly affect the tau protein or AD progression (Table 2., Figure 6).

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No	ID	Name	Amplitude	S/N	Frequency	Effect on tau protein/AD
1	DB01012	Cinacalcet	0.40481	13.45278	0.080	Indirect on tau phosphorylation
2	DB01393	Bezafibrate	0.32723	9.6733	0.080	Reduces Aβ and tau pathology
3	DB06287	Temsirolimus	1.14833	28.35191	0.167	Reducing tau hyperphosphorylation
4	DB01590	Everolimus	3.23776	20.74836	0.167	Reducing tau hyperphosphorylation
5	DB00035	Desmopressin	2.30313	20.55908	0.167	Could influence Aβ/tau cross-interactions
6	DB01130	Prednicarbate	1.34609	18.58936	0.167	Potential tau aggregation modulator
7	DB01656	Roflumilast	17.85085	0.05962	0.167	Ameliorates cognitive deficits in tauopathy models
8	DB00166	Lipoic acid	17.1443	0.04087	0.167	Reduces tauopathy
9	DB01420	Testosterone Propionate	1.00595	22.21979	0.194	Hyperphosphorylation of tau
10	DB06772	Cabazitaxel	2.47515	22.12693	0.194	Microtubules stabilisation
11	DB01599	Probucol	0.50271	19.55832	0.194	Reduce amyloid deposition
12	DB08866	Estradiol valerate/Dienogest	0.91072	19.02854	0.194	Prevents tau hyperphosphorylation
13	DB00850	Perphenazine	0.71948	14.65717	0.333	Lower the levels of insoluble tau.
14	DB06699	Degarelix	2.05541	13.78509	0.333	Hormone modulation may influence neurodegeneration.
15	DB00883	Isosorbide Dinitrate	0.87453	22.00889	0.342	Nitric oxide modulation (could influence neurodegeneration)
16	DB00243	Ranolazine	1.48002	23.2534	0.435	Reduces oxidative stress, lacks tau-specific evidence.e
17	DB00423	Methocarbamol	0.80441	21.58701	0.435	Promoting tau clearance
18	DB01136	Carvedilol	1.07707	21.14359	0.435	May reduce Aβ and tau toxicity
19	DB00206	Reserpine	2.15401	19.96937	0.435	Reduces Aβ toxicity

Table 2. The list of identified Drugbank compounds from the tau protein ISM spectrum

Cinacalcet was reported to play a significant role in AD. As a calcimimetic agent used for hyperparathyroidism, it modulates calcium-sensing receptors, which may influence amyloid-beta (Aβ) pathology and neuronal calcium dysregulation, both implicated in AD. It may indirectly influence tau phosphorylation by regulating calcium signalling, but direct evidence is lacking.[48] Bezafibrate is a PPAR-alpha agonist used for lipid disorders. It has been shown that bezafibrate treatment could attenuate the severity of tau pathology in the streptozotocin-intracerebroventricular-induced sporadic AD rat model.[49] Temsirolimus[50][51] and Everolimus[52][53] were reported to demonstrate neuroprotective effects in AD models by reducing tau hyperphosphorylation and promoting autophagic clearance of amyloid-β (Aβ) and tau aggregates, improving cognitive function. Desmopressin[54], a neurohypophyseal hormone analogue, has been suggested to modulate amyloid aggregation, though its direct role in tau pathology remains less explored. Prednicarbate[55] (a topical corticosteroid) was identified in a drug screening study as one of the prescription drugs that may influence hyperphosphorylated tau aggregation and cytotoxicity. Roflumilast[56] ameliorates cognitive deficits in AD mice by reducing Aβ and tau pathology, potentially via nitric oxide signalling and upregulating Aβ transporters like ABCB1. Lipoic acid[57][58][59][60][61][62][63][64][65][66] shows potent antioxidant and anti-inflammatory properties, mitigating tau hyperphosphorylation, oxidative stress, and behavioural deficits in tauopathy models, while enhancing mitochondrial function. Testosterone Propionate is a synthetic androgen, and androgens are found to regulate tau phosphorylation.[67] Cabazitaxel is a chemotherapy agent. While not directly linked to AD, its ability to stabilise microtubules has prompted interest in its potential to address tau pathology, a hallmark of AD.[68] Probucol is a lipid-lowering drug with antioxidant properties. It may reduce oxidative stress and amyloid deposition, which are implicated in AD.[69] Estradiol valerate and Dienogest are hormonal agents. Estrogen has been studied for its neuroprotective effects, preventing neural tau hyperphosphorylation, particularly in postmenopausal women, who are at higher risk for AD.[70] Perphenazine is an antipsychotic, found, among some others, to lower the levels of insoluble Tau.[71] Degarelix is a GnRH antagonist. Hormonal modulation has been explored in AD, particularly regarding sex hormones and their impact on cognitive function.[72] Isosorbide Dinitrate is a vasodilator. Improving cerebral blood flow may have neuroprotective effects in AD.[73] Ranolazine is an antianginal

drug. It modulates cellular metabolism and has been explored for its potential to enhance neuronal energy deficits in AD.[74] Methocarbamol[75], a carbonic anhydrase inhibitor, has been shown to reduce tau toxicity by promoting its clearance. Studies in tauopathy models, including zebrafish and transgenic mice, have demonstrated that methocarbamol can rescue neuronal degeneration, improve cognitive function, and reduce phosphorylated tau levels. Carvedilol is a beta-blocker with antioxidant properties. It may reduce oxidative stress and inflammation, both implicated in AD.[76] Reserpine is an antihypertensive with neuroprotective potential. Reserpine[77][78], in particular, has been studied for its ability to modulate neurotransmitter systems involved in AD. The complete list of the Drugbank candidates is given in the Supplementary Material.

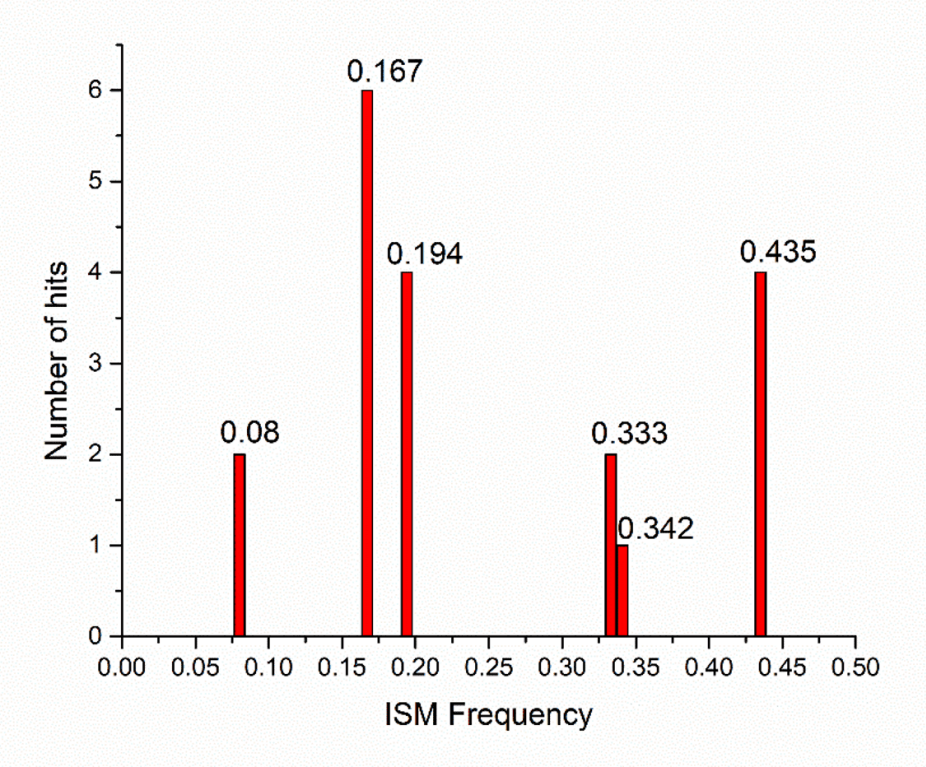

Figure 6. Distribution of Drugbank hit compounds at different ISM frequencies

Although not directly involved in interaction with tau protein, the identified drugs affect processes in AD via other targets in the tau signalling pathway, such as phosphorylation. This may be possible due to the PPI interactions in the signalling pathways, occurring at the standard ISM frequency. This suggests the method might capture broader signalling relationships, potentially reflecting *in vivo* activity, although the mechanism for this requires further investigation and validation.

An important future direction would be integrating AI tools, particularly machine learning and deep learning models, to automate and refine the identification of druggable motifs within IDPs. AI could be employed to predict binding hotspots and rank compound libraries based on learned bioactivity patterns, potentially reducing the need for extensive experimental validation. Fusing traditional structure-based modelling and AI-assisted screening might offer a more robust platform for targeting IDPs.

3.2. Coconut database candidates

Compounds from the Coconut database were also screened on all frequencies, as were the Drugbank compounds. However, in the case of small organic molecules, contrary to the proteins, mere calculation of A and S/N values cannot be considered the final step. Further insight into the candidate's structure and properties is required. Therefore, the dS values of the candidates were also calculated. Those values were integrated into the final Total Score descriptor as their product, and the candidates were finally sorted accordingly. The top compounds at all frequencies are presented in Table 3.

Compound ID	Amplitude	S/N	Drug Score	Total Score	F
CNP0504067.0	2.69306	27.51457	0.423587	11.65481	0.080
CNP0126636.1	2.04621	22.20786	0.387137	8.597492	
CNP0126636.2	2.04621	22.20786	0.387137	8.597492	
CNP0560502.0	0.94469	20.84645	0.403955	8.421033	
CNP0195295.1	1.73741	22.269	0.373435	8.316027	
CNP0195295.2	1.73741	22.269	0.373435	8.316027	
CNP0195295.3	1.73741	22.269	0.373435	8.316027	
CNP0532732.0	0.5916	16.87571	0.461918	7.795201	
CNP0581434.0	0.914	18.88449	0.411552	7.771957	
CNP0111317.1	1.08345	21.70098	0.350324	7.602369	
CNP0266316.1	4.27897	33.72198	0.384931	12.98064	0.167
CNP0168057.1	**4.98946**	**35.85598**	**0.345029**	**12.37137**	
CNP0427543.1	3.50803	37.84521	0.318055	12.03686	
CNP0135438.1	3.02339	39.19001	0.300212	11.7653	
CNP0327834.1	2.50137	36.20926	0.322438	11.67525	
CNP0297394.1	3.15932	31.3039	0.355757	11.13657	
CNP0297394.2	3.15932	31.3039	0.355757	11.13657	
CNP0359990.1	3.04876	34.43667	0.316433	10.8969	
CNP0449680.1	6.37951	30.71995	0.35436	10.88594	
CNP0072358.1	1.56251	29.1654	0.373213	10.8849	
CNP0267855.1	7.15267	54.76951	0.341619	18.71031	0.194
CNP0267855.2	7.15267	54.76951	0.341619	18.71031	
CNP0115161.1	5.96389	48.03602	0.338886	16.27874	
CNP0271940.1	6.0078	47.16665	0.332809	15.6975	
CNP0144759.1	3.8288	32.95079	0.463956	15.2877	

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Compound ID	Amplitude	S/N	Drug Score	Total Score	F
CNP0144759.2	3.8288	32.95079	0.463956	15.2877	
CNP0144759.3	3.8288	32.95079	0.463956	15.2877	
CNP0399889.1	5.28587	42.04294	0.338886	14.24777	
CNP0399889.2	5.28587	42.04294	0.338886	14.24777	
CNP0271195.1	5.09783	39.06683	0.332809	13.00181	
CNP0425508.1	8.05391	41.71905	0.386084	16.10707	0.333
CNP0426456.1	7.35555	40.40947	0.382365	15.45116	
CNP0580557.0	79.88268	108.701	0.120457	13.0938	
CNP0492610.1	6.6601	45.59386	0.265522	12.10616	
CNP0574550.1	4.46071	24.83686	0.482007	11.97155	
CNP0493035.1	4.37475	24.46051	0.482007	11.79014	
CNP0598400.0	5.28339	29.81018	0.391643	11.67494	
CNP0571478.1	5.53395	38.77414	0.265522	10.29537	
CNP0491847.1	5.6254	39.18781	0.255595	10.01621	
CNP0357360.0	7.96913	39.01198	0.255595	9.971255	
CNP0285895.1	8.68994	43.58418	0.387729	16.89884	0.341
CNP0313376.1	8.34496	42.95288	0.370435	15.91127	
CNP0578185.1	6.39618	32.0514	0.443659	14.21988	
CNP0291861.1	6.25785	39.10642	0.350141	13.69275	
CNP0538593.1	3.96833	33.60902	0.403861	13.57337	
CNP0180487.0	5.74512	36.78961	0.362787	13.34681	
CNP0525297.1	6.12899	37.81863	0.35206	13.31442	
CNP0525297.2	6.12899	37.81863	0.35206	13.31442	
CNP0525297.3	6.12899	37.81863	0.35206	13.31442	
CNP0525297.4	6.12899	37.81863	0.35206	13.31442	

Compound ID	Amplitude	S/N	Drug Score	Total Score	F
CNP0551487.1	16.05786	80.20866	0.238926	19.16397	0.435
CNP0199424.0	6.89854	48.91952	0.312082	15.26689	
CNP0509389.2	3.92528	34.60863	0.434754	15.04625	
CNP0105199.1	15.39043	73.53799	0.199091	14.64072	
CNP0417346.0	4.44435	36.20801	0.395972	14.33737	
CNP0048849.1	26.47273	80.3243	0.174197	13.99222	
CNP0061932.1	2.42416	38.15904	0.365668	13.95353	
CNP0151916.0	2.41066	37.60989	0.349215	13.13393	
CNP0078724.1	20.89881	53.48316	0.244763	13.09071	
CNP0078724.2	20.89881	53.48316	0.244763	13.09071	

Table 3. The top ten compounds from the Coconut database, at all frequencies, ranked by Total Score. Bryostatin-14 is marked in bold.

doi.org/10.32388/5DGYBH.2

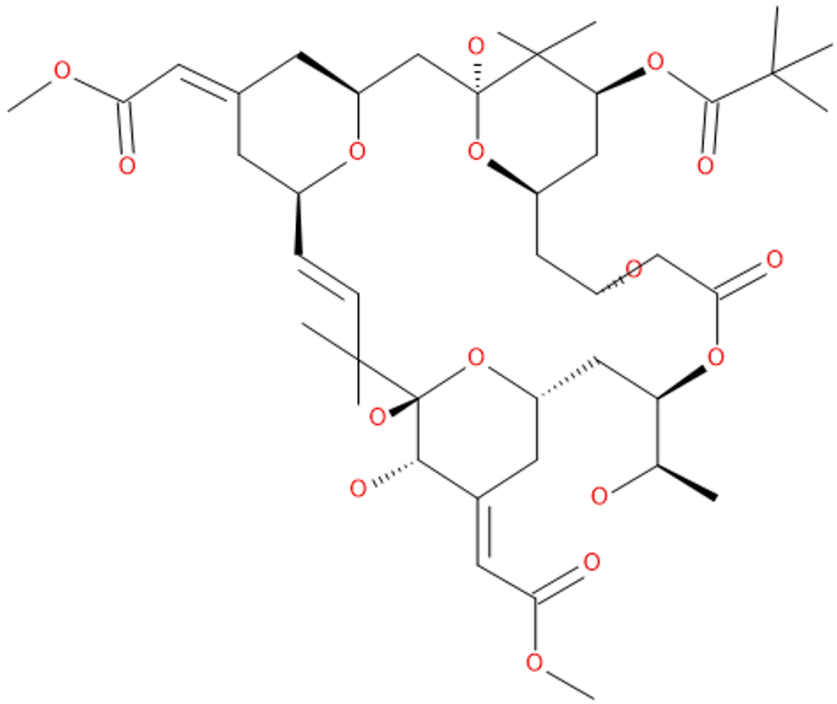

Figure 7. Structure of Bryostatin 14.

We identified a highly ranking compound, bryostatin-14, at F(0.167) (Figure 7). Bryostatins are macrocyclic lactones from marine bryozoans. They are increasingly being considered for therapeutic development in AD because of their ability to modulate protein kinase C (PKC) activity and ultimately mitigate pathological features of AD, such as tau hyperphosphorylation and amyloid-β (Aβ) aggregation. Several preclinical studies have demonstrated that bryostatin-1 enhanced synaptic plasticity and cognitive behaviour through the activation of PKCε, which inactivated glycogen synthase kinase-3β (GSK-3β), a critical kinase driving tau hyperphosphorylation[79][80]. The inhibition of GSK-3β reduces pathological aggregation of tau, which improves neurons' survival in transgenic tauopathy models[79]. Although their binding to tau protein is not well characterised, their modulation of tau phosphorylation through PKC/GSK-3β signalling makes them an unusual therapeutic approach to targeting tauopathies such as AD. Interestingly, bryostatin-14 is a hit at F(0.167), the most populated F among Drugbank compounds (Figure 6).

doi.org/10.32388/5DGYBH.2

3.3. Comparison to the Martini-IDP forcefield

In the recent paper by Wang et al[19], the Martini forcefield was updated with the parameters for small molecules. The authors carried out 15us molecular dynamics simulations for the validation. Their study examined the contact frequency between the small molecule–ligand and the IDP. The systems studied were Alpha synuclein, p53, and Androgen receptor, as well as their corresponding ligands (reference[19], Figure 3 A-C). The corresponding domains with the highest contribution to protein-ligand ISM-SM CS frequencies (Figure 8), and their comparison to the Martini-IDP forcefield, are presented in Table 4.

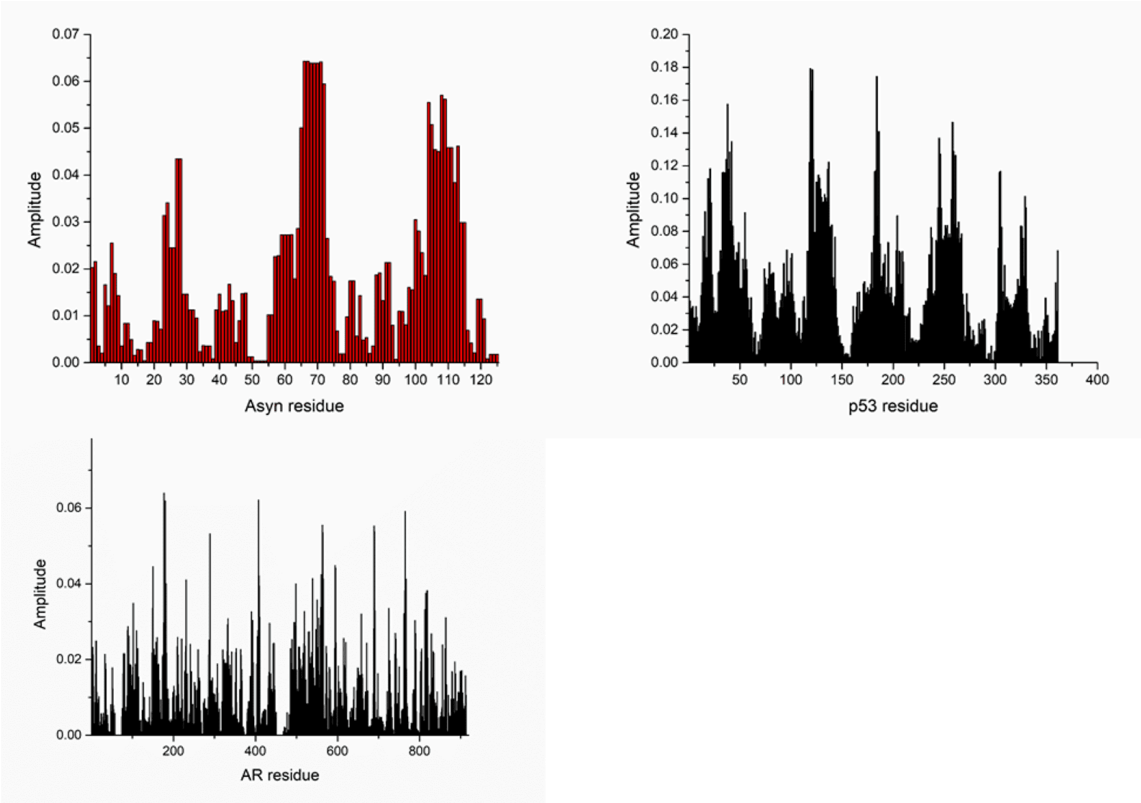

Figure 8. Corresponding amino acid residues in the alpha-synuclein, p53 and adrenergic receptor, at their CS frequencies with the ligands

doi.org/10.32388/5DGYBH.2

System	Martini-IDP	ISM-SM region
alpha-synuclein-fasudil	**3**, 38, **93**, **115**, 124, 127, 135	**1, 2**, 7, 23, 24, 27, 28, 66, 67, 68, 69,70, 71, **104**, 108, **109** (Slide window width 17)
p53 - Ligand 1050	**23**	**21**, 38, 119, 184, 258, 305, 329, 361 (Slide window width 33)
AR - EPI-002	396, **405, 406**, 432, 433, 437, 438	177, 289, **407**, 563, 689, 765 (Slide window width 8)

Table 4. The key residues for IDP-ligand interaction were compared from MD simulations with the Martini-IDP forcefield and the ISM-SM method. The residues most approximately identified in both methods are in bold.

The results demonstrate that ISM-SM complements traditional forcefield-based approaches, offering alternative insights into IDP-ligand binding regions. The observed overlaps support ISM-SM's validity, while the method's broader residue selection suggests it could be particularly valuable for capturing dynamic, transient interactions—hallmarks of intrinsically disordered proteins.

3.4. Comparison to ensemble docking results

A similar study on the ensemble docking of three compounds to alpha-synuclein has been reported recently[7]. Fasudil, Ligand 47 and Ligand 23 were docked to a-synuclein conformations obtained from the MD simulations. According to the results, the aminoacid residues with the highest probability for interaction with Ligand 47 are in the domain 121-139, with the top values at Y133 and E137. According to the ISM-SM analysis, carried out similarly to paragraph 3.3, domain 70-134 at F(0.226) was detected as the most responsible for the protein-ligand interaction.

4. Limitations of the method

This study primarily utilises the ISM-SM method, an in silico approach based on electron-ion interaction potentials, to identify potential tau modulators. A key limitation is that ISM-SM predicts potential interactions based on calculated spectral compatibility rather than direct structural binding or dynamics. The underlying biophysical mechanism linking EIIP frequencies to specific long-range interactions and

biological activity warrants further theoretical and experimental investigation, especially for dynamic IDPs like tau. The validation here relies heavily on identifying known drugs or compounds with literature support for indirect effects on tau pathology or AD, rather than primary experimental validation of direct binding or modulation of tau by the novel candidates identified (e.g., from the Coconut database). Furthermore, the study does not directly compare ISM-SM's performance against other established computational methods for IDP ligand discovery, such as enhanced sampling MD simulations or ensemble docking approaches. While interesting, the potential of ISM-SM to identify compounds acting indirectly through pathway interactions also introduces challenges in confirming the mechanism of action and requires careful interpretation. Future work should incorporate experimental validation (e.g., binding assays, cellular assays) and comparative computational studies to more rigorously assess the predictive power and applicability of ISM-SM for IDP drug discovery.

5. Conclusion

Intrinsically disordered proteins (IDPs) remain the most problematic targets for drug discovery because of their high dynamics and poorly defined binding pockets. Conventional structure-based approaches are generally ineffective in finding effective small-molecule modulators of such proteins. The Informational Spectrum Method for Small Molecules (ISM-SM) is another method that uses long-range interactions to predict functional ligand interactions by the electron-ion interaction potential (EIIP). We depicted our study to demonstrate that ISM-SM is able to accurately identify small molecules that potentially have activity against tau protein, which is an essential contributor to Alzheimer's pathology. Based on their spectral compatibility, the drug candidates identified by this study were considered likely direct/indirect modulators of tau. In fact, because of PPI, ISM-SM can also identify drugs that indirectly impact the target through other signalling pathways, such as a singular *in vivo* activity on a similar disease related to the target. This is a disadvantage to the target itself, although it can be considered an advantage from a potential in vivo activity standpoint. Combining ISM-SM with molecular dynamics simulation and experimental validation could further improve the efficiency and precision of IDP-targeted drug discovery.

Integrating ISM-SM with molecular dynamics simulations and experimental validation could significantly enhance the efficiency and accuracy of IDP-targeted drug discovery. This approach accelerates the identification of novel candidates and reduces the high costs associated with traditional high-throughput screening methods. Given its success in other areas of drug repurposing, ISM-SM

stands as a valuable tool for advancing therapeutic development, not only speeding up the identification of novel candidates but also aiding in identifying IDPs implicated in neurodegenerative disorders and other complex diseases. Future work should focus on refining ISM-SM-based predictions through experimental validation and exploring its applications for broader IDP-related disease targets.

AI methods could significantly enhance the framework provided, expediting the process of identifying small-molecule modulators of IDPs with higher accuracy. This would have a transformative effect on the drug discovery pipelines for this challenging class of proteins.

Supplementary Materials

Table S1 contains the lists of Drugbank and Coconut database candidates at all ISM frequencies, along with the amino acid and atomic group EIIP parameters used in the calculations.

Statements and Declarations

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Data Availability

This article and the Supplementary Material include the original contributions presented in this study. Further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Statement on AI Use

Artificial intelligence tools were used solely to improve the clarity and language of the manuscript. All scientific content, analysis, and conclusions are the author's original work.

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