ISSN 0974 - 9373

Vol. 26 No. 4 (2022) **J. Int. Acad. Phys. Sci.** pp. 471-482

Computational ADME Analysis and Antioxidant Potential of 5-methoxy-2-methyl-1H-indol-3-yl)-N'-(substituted benzylidene) Acetohydrazide Derivatives for Neuroprotection

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(Received October 11, 2022, Revised November 7, 2022, Accepted November 21, 2022)

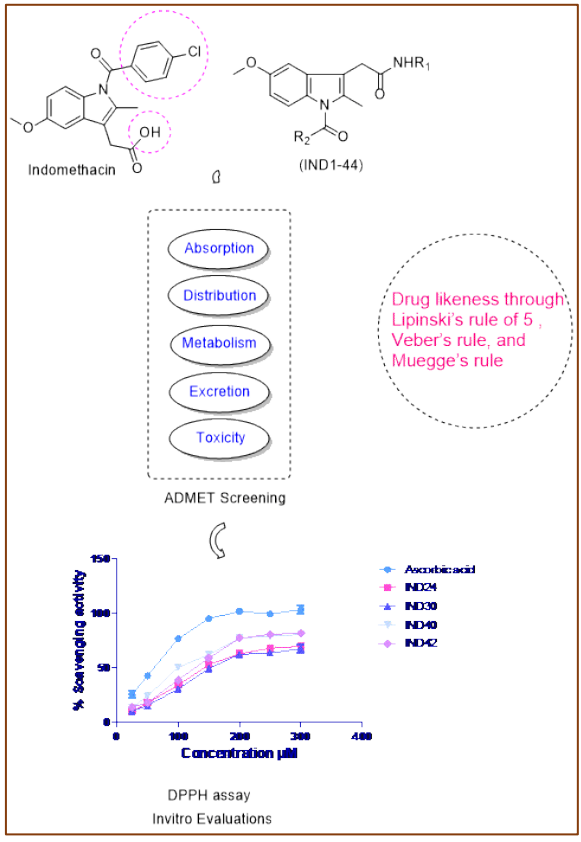
Abstract: Oxidative stress is a constant threat to human health because it causes an excessive production of free radicals, most notably reactive oxygen species, which are toxic to DNA, RNA, and proteins. The accumulation of free radicals in humans has been linked to cancer, inflammation, atherosclerosis, Alzheimer's disease, Parkinson's disease, and the ageing process. Antioxidants have been shown to reduce oxidative damage and lower the chance of developing chronic diseases, earning them the term "free radical scavengers" of the human body. It is possible to use indoles, which are heterocyclic compounds, in treatments since they are antioxidants. In the last few years, new approaches have been developed using indole derivatives to counteract the damage produced by free radical generation.

The goal of this investigation was to use the indole scaffold to create novel antioxidant candidates. In this investigation, A new series of 5-methoxy-2-methyl-1H-indol-3-yl)-N'- (substituted benzylidene) acetohydrazide derivatives were produced in good yields in an effective manner. The elemental analysis and spectrum data completely characterized all of the substances. Using a computational method, we predicted these analogs' binding modalities and pharmacokinetic properties. The biological activities of these substances were predicted using the ADMET prediction programme Molinspiration before any

experimental lab activity could begin. The DPPH technique then measures antioxidant activity. In addition, the DPPH assay confirmed that all of the samples had antioxidant properties that were superior to those of ascorbic acid. The highest levels of antioxidant activity were seen for a compound that had a para-dimethylaminophenyl substitution. An in silico ADMET, Swiss ADME, oxygen scavenging potential assisted to derive effective neuroprotective for the present study.

Keywords: Alzheimer's Disease, In-Silico, DPPH, indole analogs, oxygen scavenger.

Graphical Abstract



1. Introduction

Heterocyclic synthesis is a powerful organic synthesis approach for developing novel drugs[1-3]. Nitrogen-containing heterocyclic molecules provide highly functionalized scaffolding for effective and selective medicines[4-6]. Oxidative stress is clinically relevant in numerous disease processes, the most prevalent of which are Alzheimer dementia (AD), Lambert-Eaton myasthenic syndrome (LEMS), and myasthenia gravis (MG).

The most major neurotransmitter system implicated in the aetiology of Alzheimer's disease is the cholinergic group of neurons, which is involved in cortical activity, cerebral blood circulation, memory and learning-oriented processes, and modulation of cognition[7].

Antioxidants from natural sources, which are frequently incorporated into dietary practises and are generally favoured to battle oxidative stress, can help postpone the start of AD and slow its progression. This strategy hasn't, however, been thoroughly researched. Additionally, there is mounting proof that the pathophysiology of AD may be better addressed by combining antioxidants with a nutrient-rich diet[8]. Several research studies have been conducted highlighting the effect of antioxidants in AD (Figure 1).

Indomethacin is a crucial scaffold in the search for possible Alzheimer's medications[9]. Azam et al. have conducted extensive research on 24 non-steroidal anti-inflammatory medications (NSAIDS) of eight distinct groups to identify prospective therapy options for reducing amyloid damage in Alzheimer's disease[10]. In addition, they made observations and came to the conclusion that NSAIDs that contain an aryl or heteroaryl aromatic moiety connected by a linker consisting of one to two atoms to a distal aromatic group satisfy the structural requirements for contact. According to earlier studies, the hybridization of indomethacin and tacrine increases the interaction with cholinesterase in both CAS and PAS. This improvement is due to an indole ring that is present in the structure of both molecules[11].

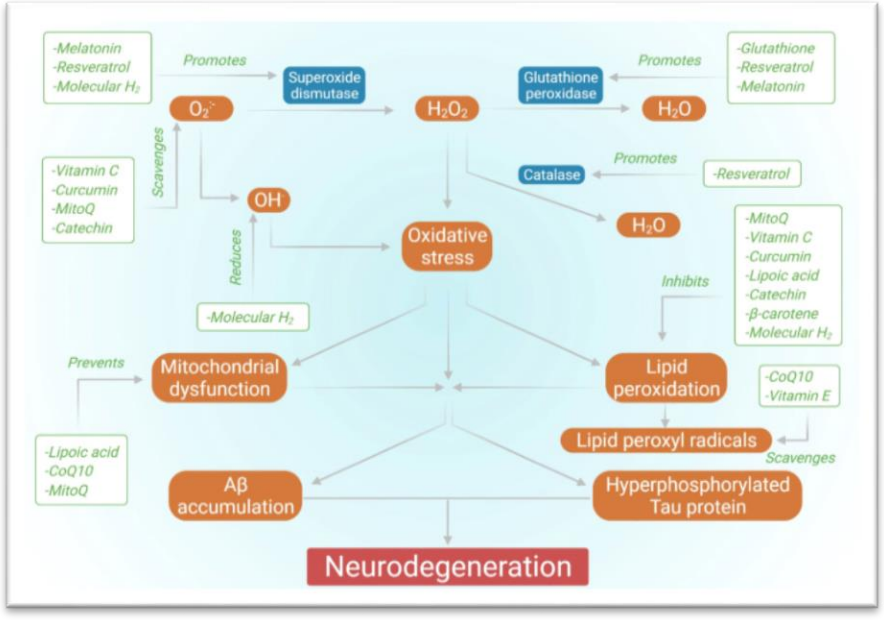


Figure 1: Effect of various antioxidants in countering AD

In the present paper, we have reported the ADME screening, in vitro antioxidant and AChE activity of indomethacin derivatives (Table-S1). Potential of a novel molecule is often first examined by virtual tools in modern drug discovery process. A compound's molecular structure can provide insight into whether or not it will demonstrate beneficial therapeutic activity (also known as "drug-likeness")[12,13]. Before synthesis, it is essential to predict bioavailability and bioavailability-related parameters, such as solubility and lipophilicity. This may be the most effective way to avoid chemical depletion, wasted time, and potential ecological damage may be best accomplished in this way. As an added measure, we analysed each compound's ADMET profile.

2. Materials and Methods

2.1. ADME analysis

The drug-likeness properties of the 44 indomethacin analogues were calculated using the Swiss ADME database[14], the admet SAR-2.0 online webserver (http://lmmd.ecust.edu.cn/admetsar2/), and the Molinspiration server (http://www.molinspiration.com/)[15]. Physiochemical characteristics, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and toxicity investigations are among the projected outcomes. Molecular weight (MW), number of rotatable bonds (NRB), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), molar refractivity (MR), and topological surface area are among the physicochemical parameters used for hits screening (TSA). Lipophilicity and solubility are the other two key criteria that are investigated for potential medication development. The hits are categorized as insoluble if their solubility qualities fall outside of this range[16]. Three well-known approaches were used to evaluate distinct pharmacokinetic parameters for hits: Lipinski's rule of 5[17], Veber's rule, and Muegge's rule[18] . The oral bioavailability of the putative active analogues was determined using Lipinski's rule of five and Veber's guidelines. Muegge's rule determined whether a chemical had a good chance of becoming a drug candidate. The most recent version of the admetSAR-2.0 online tool was used to forecast toxicology.

2.2 DPPH radical scavenging assay

The scavenging activity of the stable DPPH free radical served as the basis for the measurement of the antioxidant activity. When it reacts with antioxidants, the free radical known as 2,2-diphenyl-1-picrylhydrazyl, which has a purple tint, produces the yellow-colored compound known as diphenylpicrylhydrazine. The capacity of a substance to scavenge free

radicals is evaluated using this assay. This capacity is measured as a result of the DPPH being reduced by an antioxidant. The assay was carried out according to the protocol[19–21] that was discussed earlier. In a nutshell, in a 96-well plate, 50 and 100 M (10 L) of the synthesised compounds were combined with 20 L, 10 mM (stock in methanol) of DPPH (Hi-Media). The buffer used for the synthesis was Tris-HCl, and the pH was 7.4. Methanol was used to bring the volume of the solution up to its final value of 200 L. The 96 well plate was kept in an incubator at 37 degrees Celsius for 25 minutes while it was shaken moderately in a water bath. At a wavelength of 520 nm, absorbance was determined to be present in the reaction mixture. The reduction percentage (RP) of the DPPH was calculated using the equation RP = 100 [(A0 - Ac)/A0], where A0 represented the absorbance of the control, which was untreated DPPH, and Ac represented the absorbance of the test, which was treated with DPPH. In addition, seven different compounds were chosen for further investigation based on their capacity to scavenge free radicals. Analogs of indomethacin were tested at seven different concentrations (25, 50, 100, 150, 200, and 250 M). The results were analysed. During the DPPH test, ascorbic acid served in the capacity of the standard. The experiment was carried out three times to ensure accuracy[22].

3. Results

3.1 Physiochemical properties, ADME/T and drug-likeness properties

Early forecasts of physiochemical characteristics, ADME/T, and drug-likeness properties can be made using in silico tools like SwissADME and the admet SAR-2.0 website. Lipinski's rule of five and Veber's rule were used to calculate the oral bioavailability of the potential active substances. Muegge's rule used the pharmacophore point calculation to evaluate the likelihood of a chemical becoming a successful drug molecule [17]. Lipinski's, Veber's, and Muegge's rules were followed by the analogues IND, IND -4, IND-5, IND-6, IND-9, IND-14, IND -15, IND-18, IND-40, and IND -42. On the other hand, IND-22, IND- 24, IND-25, IND-30, and IND-31 followed just Veber's and Muegge's rules, and in accordance with these standards[6], it was anticipated that finished compounds would have a high bioavailability and fulfil the criterion for drug likeliness. In addition, excluding IND-20, IND-21, IND-26, and IND-44, forty analogues have a high absorption rate in the human intestine, whereas four molecules have a low absorption rate. Log S scale projected the moderate solubility level of 10 analogues (IND, IND-4,5,6, 9, 14,15,18, 40, 42), and IND-20, IND-21, and IND-26 have AMES hazardous nature. Log S scale also predicted that six analogues (IND, IND-1,2,3,16,17)

were carcinogenic and fell under the 'DANGER' category. IND-40 and IND-42 are solvents that dissolve in moderate amounts and pose a manageable risk (Table-1). The Molinspiration service provides an estimated drug-likeness model score for recently created analogues of indomethacin here (Figure-2).

Table-1: The physiochemical, lipophilicity, water-solubility, pharmacokinetics, drug likeliness, and toxicity predictions of IND-24, IND-30, IND-40 and IND-42

	SwissADME												admet SAR 2.0				
	Physicochemical properties						lipophilicity	Water Solubility	Pharmakokinetics	Drug Likeliness			Toxicity Prediction				
Analogue	Mwt	nrotb	HBA	HBD	MR	TPSA (A)	Consensus Log Po/w	class	GI absorption	Lipinski	Veber	Muegge		AMES	Acute oral	Carcinoge	Rat Acute
IND-24	475.92	8	5	2	132.35	92.92	4.3	Poorly soluble	High	0	0	1	Weak	Non-	III	Non-	2.9162
IND-30	475.92	8	5	2	132.35	92.92	4.3	Poorly soluble	High	0	0	1	Weak	Non-	III	Non-	2.9162
IND-40	491.92	8	6	3	134.38	113.15	4.01	Moderately Soluble	High	0	0	0	Weak	Non-	III	Non-	2.8484
IND-42	491.92	8	6	3	134.38	113.15	3.94	Moderately Soluble	High	0	0	0	Weak	Non-	III	Non-	2.8319

3.2 Antioxidant activity evaluation:

The DPPH test is a dependable method for determining whether or not substances have an active oxygen scavenging property. In order to determine whether or not the molecules that were produced possessed antioxidant properties, they were tested. The indole derivatives IND-24, IND-30, IND-40, and IND-42 all exhibited strong antioxidant activity. In comparison to the other molecules in this series[22], those that include donor groups of the -OH type are significantly more effective oxygen scavengers. The DPPH reduction potential of the remaining molecules was found to be adequate (figure 3).

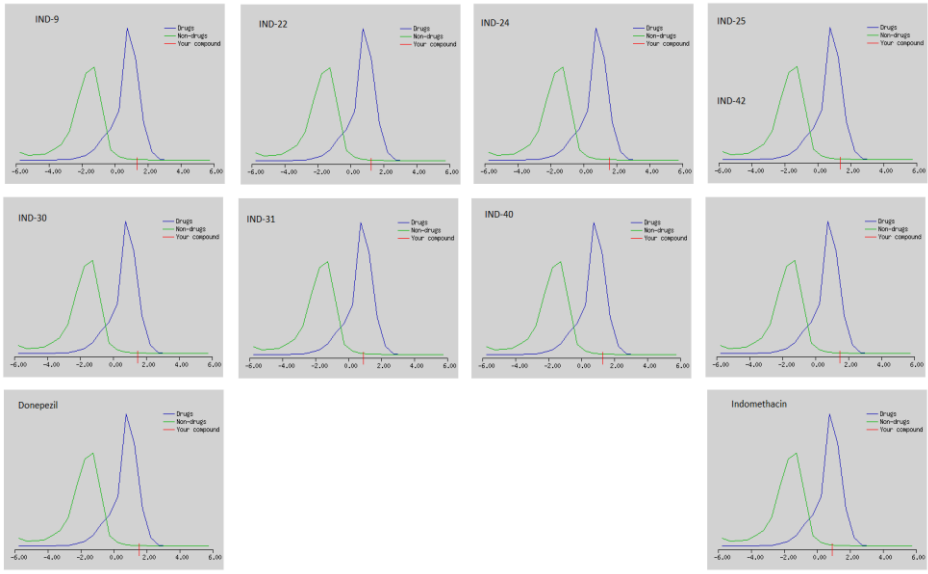


Figure-2: Drug-likeness model score by Molinspiration server of newly designed indomethacin analogues, indomethacin and Donepezil, a standard anti-cholinesterase drug. Positive score for any query compound indicates its drug potential

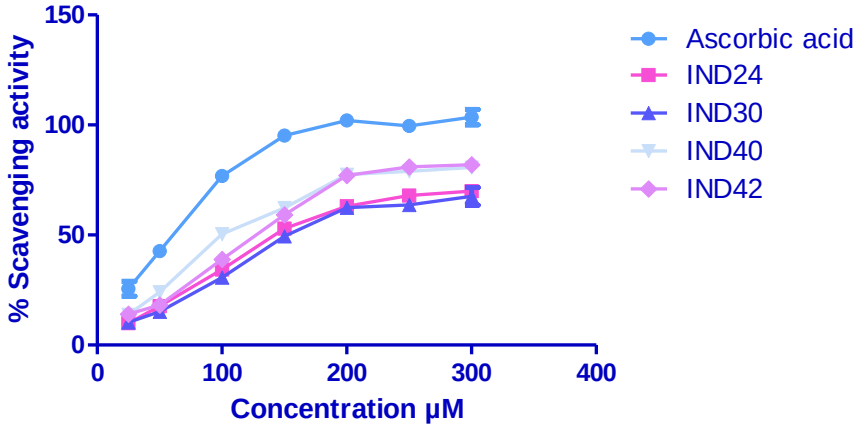


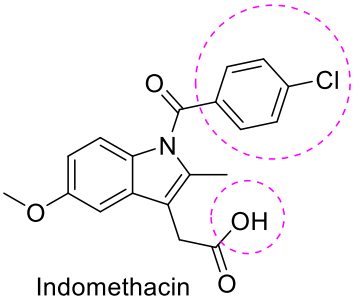
Figure 3: DPPH based free radical scavenging activity of synthesized compounds.

4. Discussion

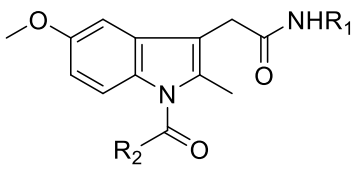
In this publication, the in silico ADME and in vitro antioxidant activity of a new series of indole derivatives have been described. The indole derivatives were screened using computational methods. Good anti-oxidant activity for neuroprotection can be achieved by combining substituted benzaldehyde with

an indole pharmacophore and ensuring that the compound possesses a suitable number of hydrogen donor–acceptor sites.

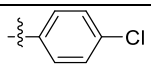
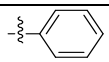
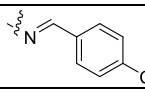
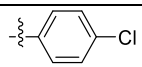
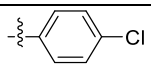
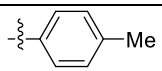
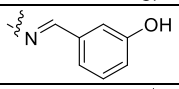
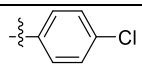
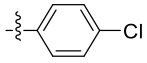
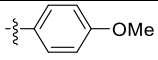
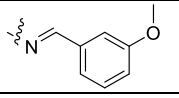
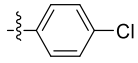
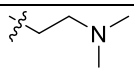
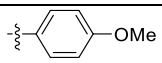
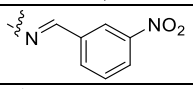
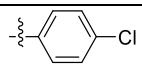
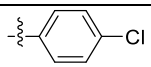
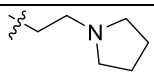
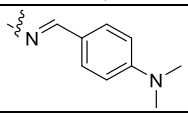
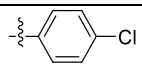
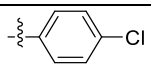
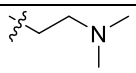
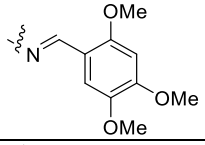
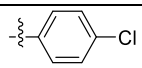
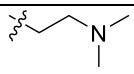
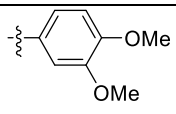
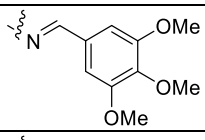
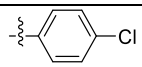
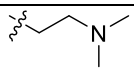
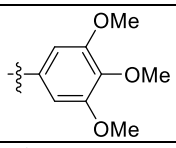
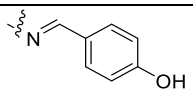
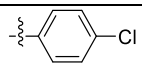
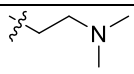
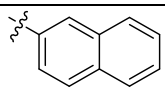
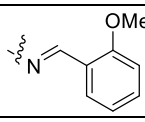
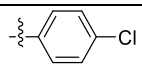
Table S1. List of linkers (electron donor and electron acceptor) used at R1; R2 sites of indomethacin analogues used in generating multiple analogues.

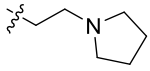
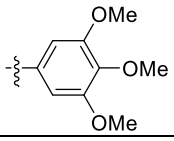
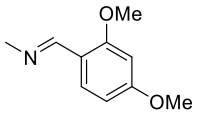
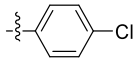
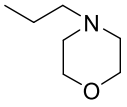
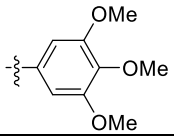
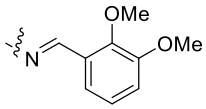
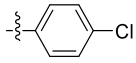
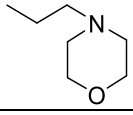
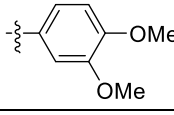
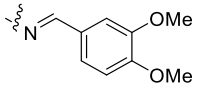
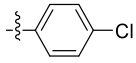
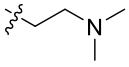
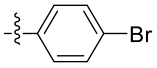
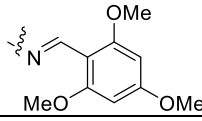
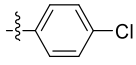
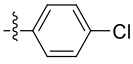
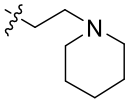
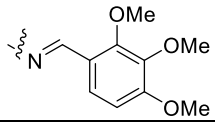
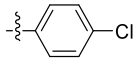
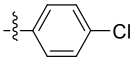
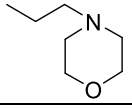
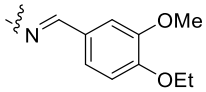
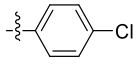
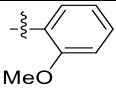
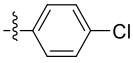
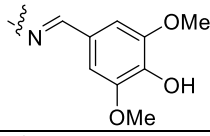
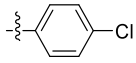
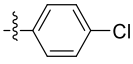
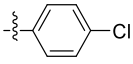
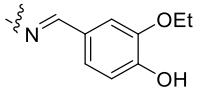
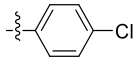
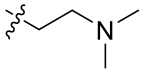
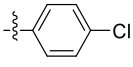
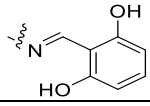
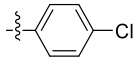
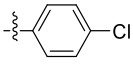
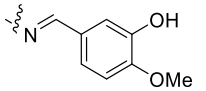
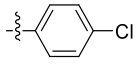


Indomethacin



(IND1-44)

	R1	R2		R1	R2
IND -1			IND -23		
IND -2			IND -24		
IND -3			IND -25		
IND -4			IND -26		
IND -5			IND -27		
IND -6			IND -28		
IND -7			IND -29		
IND -8			IND -30		
IND -9			IND -31		

IND -10			IND -32		
IND -11			IND -33		
IND -12			IND -34		
IND -13			IND -35		
IND -14			IND -36		
IND -15			IND -37		
IND -16			IND -38		
IND -17			IND -39		
IND -18			IND -40		
IND -19			IND -41		
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The results of the in silico ADME profiling, toxicity, drug-likeness, and drug scoring, along with the in vitro Antioxidant activities, revealed that the compounds are promising neuroprotective leads for the development of a selective, safe, and potent Alzheimer's disease medication.

Table 2: Indomethacin derivatives with antioxidant activity.

Sr.No	Compound	R	% Free radicle scavenging, Mean±SE (µM)	
			50	100
1	IND24	-3OH	17.68±0.478	34.34±1.333
2	IND25	-3OCH_3	11.51±0.748	27.99±1.127
3	IND30	-4OH	15.19±0.881	30.65±1.010
4	IND31	-2OCH_3	9.75±0.625	26.43±0.887
5	IND40	-2,6OH	24.10±0.889	50.39±0.930
6	IND42	-2,3OH	18.21±0.598	38.87±1.012
7	Ascorbic acid	----	42.66±1.491	76.76±0.708

5. Conclusion

The noteworthy conclusion of the study is the development of indole derivatives with substituted benzaldehyde as remarkable neuroprotective modulator for in neurovegetative illnesses such as Alzheimer's disease, more cell line research is currently being in progress with these compounds and related heterocyclic scaffolds.

Acknowledgements: We gratefully acknowledge UPES, IIT-BHU and DIT University for providing the facility to carry out all biological work.

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