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Research Article

Antioxidant potential of vanillin Schiff base hybrids: Insights from *in silico* molecular docking, ADMET, and *in vitro* DPPH assay

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Abstract

Oxidative stress is a primary driver of chronic disease. However, many synthetic antioxidants are limited by toxicity and instability. While vanillin is a promising natural scaffold, the influence of electron-withdrawing group (EWG) (i.e. halogen or NO_2) on its antioxidant efficacy remains an important area of exploration. This study aimed to synthesise and evaluate vanillin Schiff base hybrids (**1a-g**) for their antioxidant potential. The synthesised compounds were screened for radical-scavenging activity using DPPH assay. Molecular interactions were explored through docking studies against human 5-lipoxygenase (PDB ID: 6N2W) and further complemented by ADMET profiling to assess pharmacokinetic viability and adherence to Lipinski's Rule of Five. The derivatives exhibited antioxidant activity, with IC_{50} values ranging from 386.01 to 1,060.05 µM. Compound **1f** emerged as the most active (IC_{50} = 386.01 µM) among the synthesised compounds. Docking analysis revealed favourable binding affinities (-6.30 to -6.00 kcal/mol) while ADMET analysis showed that vanillin Schiff bases possess a consistent profile of oral drug-likeliness. These findings establish the vanillin Schiff base framework as a potential lead scaffold for further structural refinement. This research contributes to ongoing efforts in antioxidant development in support of the United Nation SDG 3: Good Health and Well-Being.

Keywords: condensation, electron delocalisation, hydrogen atom transfer, single-electron transfer, SwissADME

Introduction

Oxidative stress is a significant contributor to chronic diseases such as cardiovascular disorders, neurodegenerative diseases, cancer and accelerated ageing [1,2]. This is due to an imbalance between the overproduction of reactive oxygen species (ROS) and the body's ability to detoxify them. Free radicals initiate and propagate oxidative damage to living cells, including DNA, proteins, and lipids [3]. Consequently, there is a continuous and growing demand for effective antioxidant compounds that can neutralise these harmful species and mitigate their alarming threats. In this regard, research has increasingly focused on modifying natural products such as vanillin to develop novel therapeutic agents. Vanillin, a key flavour compound of the vanilla orchid, is primarily found in vanilla beans and naturally occurs in several other plant sources. It is also commonly used as a flavouring agent. Vanillin can also be obtained via a synthetic route [1]. A synthetic approach to vanillin is often preferred due to time and labour reduction for extraction [4], which enables the chemical modification of vanillin to enhance its bioactive profile.

Vanillin exhibits biological effects, including antioxidant [5], anti-inflammatory [6], and antimicrobial [7] properties. Its antioxidant activity primarily comes from the phenolic hydroxyl group (-OH) of vanillin, which can act as a hydrogen bond donor to neutralise free radicals and enhance its free radical-scavenging activity [8]. For example, papaverine, a controlled commercial drug derived from vanillin, has been used to treat asthenospermia induced by oxidative stress [9]. These properties underscore the significance of vanillin to researchers as its biological properties can be further enhanced through chemical modification. Chemical modification of vanillin has been reported to reduce oxidative stress after incorporating different nitrogen

moieties such as quinoline [10], azo (N=N) [1], hydrazone (HC=N=NH) [11] and Schiff bases (C=N) [12] (**Figure 1**). Schiff bases can be synthesised by condensation of a primary amine with an aldehyde or ketone. Schiff bases represent a versatile class of organic compounds known for their ease of synthesis and diverse biological properties such as antimicrobial [11,13], anticancer [14,15] and antioxidant [16,17] activities. Vanillin-derived Schiff bases have been reported to exhibit good antioxidant properties [12], primarily attributed to the imine (C=N) linkage. This unique structural feature can influence their electronic properties and enhance interactions with biological targets via hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms [18].

Despite extensive documentation of vanillin-derived Schiff bases as potent antioxidant scaffolds, a research gap regarding the mechanistic influence of electron-withdrawing groups (EWGs), such as halogens and NO_2 moieties, on the Schiff base moiety (C=N) remains underexplored. Current literature focuses more on electron-donating substituents to facilitate radical stabilisation [19]. EWGs can attenuate HAT kinetics while simultaneously facilitating sequential proton-loss electron-transfer (SPLET) mechanisms that enhance antioxidant properties [20,21]. Given the inherent antioxidant capacity of vanillin [8] and the broad bioactivity profile of Schiff bases [16], the synthesis of vanillin Schiff base hybrids bearing EWGs presents a promising strategy to develop novel compounds with enhanced antioxidant potential. By combining these two pharmacophores into a single molecular entity, it is hypothesised that synergistic effects could yield strong free radical-scavenging capabilities [18]. This hybridisation approach allows the strategic modulation of electronic distribution and steric hindrance to optimise the antioxidant capabilities of a compound [11].

This study aims to investigate the antioxidant potential of synthesised vanillin Schiff base derivatives. The evaluation was conducted using *in silico* molecular docking to predict binding affinities for key antioxidant enzymes, including human 5-lipoxygenase (5-LOX; PDB ID: 6N2W) [22] alongside ADMET predictions to assess drug-likeness and safety profiles. *In vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was also performed to determine their free radical-scavenging activity [23]. The findings from this comprehensive analysis provide a strong foundation for developing novel antioxidant agents with enhanced potency and safety, contributing to future therapeutic strategies against oxidative stress-related disorders.

Materials and Methods

General procedure for the preparation of vanillin Schiff bases

Compounds **1a-g** were prepared by condensation of different anilines with vanillin as described in the literature [24,25] (**Scheme 1**). An equivalent amount of aromatic primary amine and vanillin was dissolved in ethanol and stirred for 10 h at 24 °C. The reaction was monitored by thin-layer chromatography (TLC) until completion. The mixture was concentrated under reduced pressure using a rotary evaporator and the crude product was purified by recrystallisation from ethyl acetate:hexane (1:2) to obtain target compounds **1a–g** (**Figures S1–S3 and Data S1**).

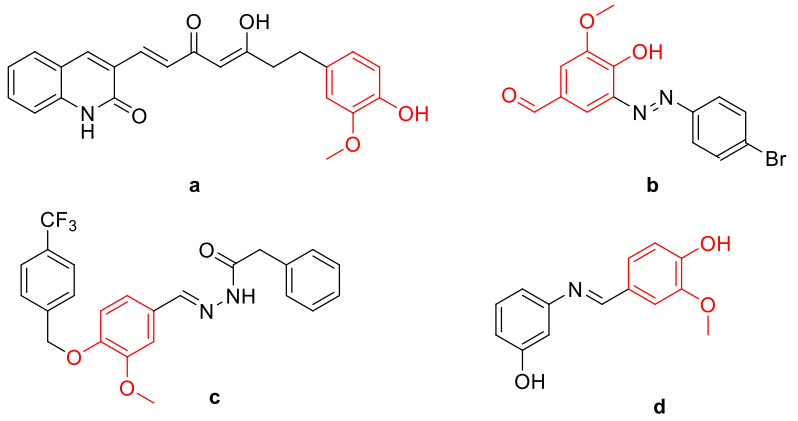
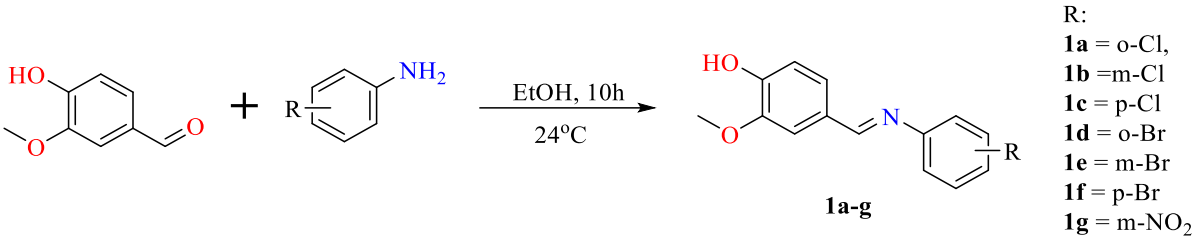


Figure 1. Vanillin derivatives containing quinoline (**A**), azo (**B**), hydrazone (**C**), and Schiff base (**D**) groups



Scheme 1. Preparation of vanillin Schiff bases (**1a–g**)

Antioxidant evaluation

The synthesised compounds (**1a-g**) were evaluated for their antioxidant potential using DPPH assay by adapting a previous method [1]. Controls used were methanol (blank) and ascorbic acid (positive). A solution of standard DPPH (0.1 mM) was added to triplicate samples at different concentrations (6.25, 12.50, 25.00, 50.00, 100.00, and 200.00 µg/mL) of the compounds. All mixtures were incubated in the dark (30 min), and subsequently measured the absorbance at 517 nm using a UV-vis spectrophotometer. The percentage of radical-scavenging activity (RSA) of the synthesised compounds was calculated based on the absorbance data following Eq. 1 (A1; blank absorbance, and A2 = sample absorbance). IC_{50} values were determined from dose–response curves generated by plotting the percentage of radical scavenging activity (RSA) against the logarithm of sample concentration. Nonlinear regression analysis using a sigmoidal model was applied to the data to calculate the IC_{50} values, and the goodness of fit was assessed by $R2 > 0.9$ for all tested compounds (Figure S4).

$$\text{Radical-scavenging activity (\%)} = \frac{A1 - A2}{A1} \times 100\% \quad (1)$$

Molecular docking analysis

The binding score of all synthesised compounds (**1a-g**) was determined by performing docking analyses (AutoDock Tools and AutoDock Vina) [26]. Protein receptor (human 5-LOX) was retrieved from the Protein Data Bank and selected as a potential therapeutic target (PDB ID: 6N2W) [22]. The grid box coordinates were positioned at coordinates x: 36.038, y: 64.982, z: 38.224. This was defined to ensure docking target on the relevant active site of human 5-LOX, which was based on the location of the co-crystallised nordihydroguaiaretic acid (NDGA) ligand [27]. The Discovery Studio® 4.0 was employed to generate receptor–ligand complexes by selecting the complex with the highest binding affinity for further analysis. The visual interpretations of receptor–ligand complex structures are available in supplementary files (**Table S1**).

ADMET study

The drug-likeness and ADMET profiles of **1a-g** were thoroughly assessed using the readily available computational platforms, SwissADME [28] and pkCSM [29]. These tools are crucial for the early identification of promising drug candidates, which effectively reduces the cost and time for trials [30]. Lipinski's Rule of Five was applied as an initial assessment for drug-likeness, whereby the predicted ADMET properties provided comprehensive insights into the pharmacokinetic behaviour of compounds **1a-g**.

Results and Discussion

Synthesis

Prior to synthesising the vanillin Schiff base hybrid, the Prediction of Activity Spectra for Substances (PASS) test was conducted to evaluate their potential as antioxidant agents (**Figure 2**) using the online tool Way2Drug. Compounds **1a–g** showed higher Pa (probability of being active) values than Pi (probability of being inactive), indicating a strong likelihood of possessing antioxidant properties [23].

Accordingly, molecular hybridisation of vanillin and various aromatic amines was performed via condensation, following previous literature [24], to synthesise vanillin Schiff bases with yields of 33.6–87.0%. The target compounds **1a–g** were successfully synthesised, and their chemical structures were elucidated through FTIR, ^{1}H, and ^{13}C NMR spectroscopy using compound **1g** bearing a nitro group to represent the formation of the vanillin Schiff base. Compound **1g** showed a disappearance of the ν(C=O) peak at 1667 cm^{-1} and a new absorption peak at 1631 cm^{-1} for ν(C=N) [31] on FTIR spectra due to the presence of imine linkage [24]. The absorption peak observed at 3402 cm^{-1} contributed to $\nu(NO_2)$. The FTIR spectrum of compound **1g** is depicted in **Figure 3**.

The formation of the target compound, with **1g** representing vanillin Schiff bases, was further evaluated using NMR analysis. The aldehyde proton at 9.87 ppm [24] disappeared, and the singlet peak that appeared at 8.61 ppm was attributed to the proton of

the imine group [11] (**Figure 4**). The proton of an imine is less downfield than that of an aldehyde because the nitrogen atom is less electronegative than the oxygen atom [32]. Meanwhile, the total aromatic proton ranging from 7.01 to 8.12 ppm in the spectra corresponded to the total expected aromatic proton of compound **1g**. The singlet peak at 3.97 ppm was attributed to the methoxy (-OCH3) proton of vanillin, whereas the singlet at 3.99 ppm corresponded to the proton of the OCH3 group of vanillin Schiff base **1g**. In the 13C NMR spectrum, the C=N and OCH3 peaks appeared at 162.5 and 55.4 ppm, respectively. The presence of aromatic carbon was indicated by a peak at 110.2–153.7 ppm, corresponding to the total expected carbon content of compound **1g.**

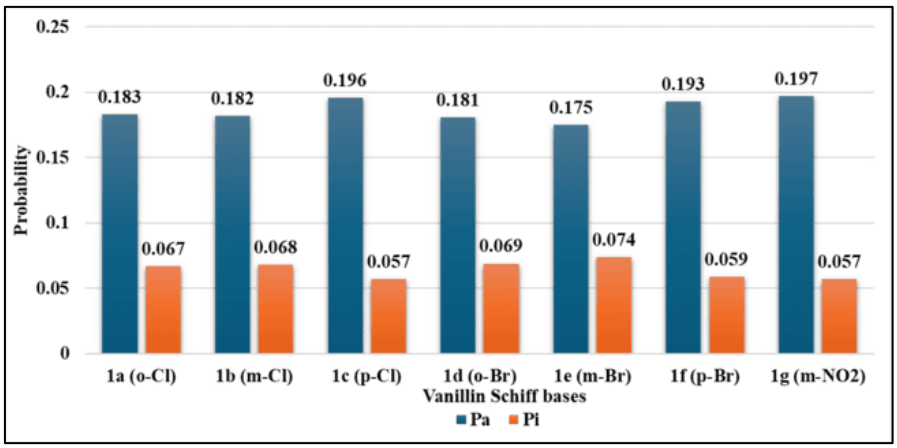


Figure 2. PASS test for vanillin Schiff bases **1a–g**

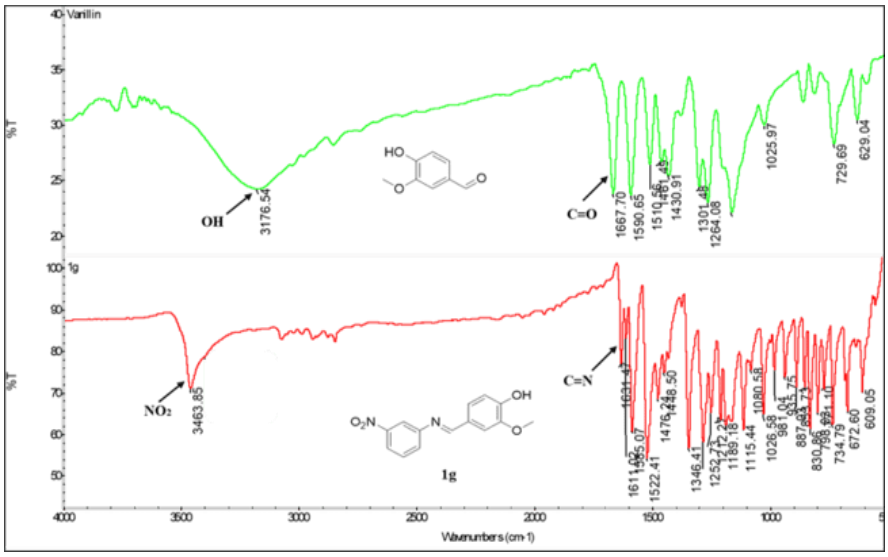


Figure 3. FTIR spectra of the vanillin precursor and vanillin Schiff base (**1g**)

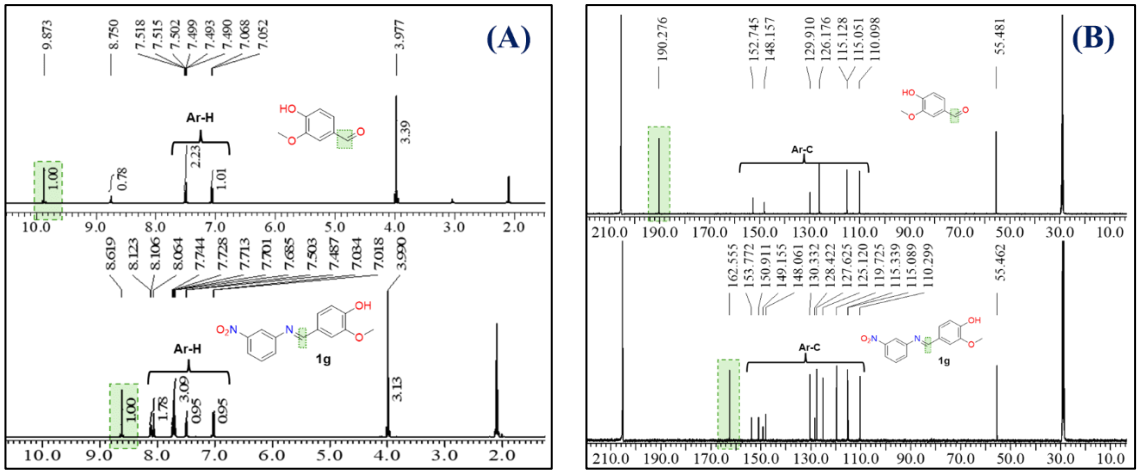


Figure 4. 1H (**A**) and 13C (**B**) NMR spectra of parent vanillin and vanillin Schiff base (**1g**)

In vitro antioxidant evaluations

Following the successful synthesis of **1a-g**, the antioxidant potential was determined via DPPH assay. Antioxidant activity was evaluated using the DPPH assay by measuring the radical-scavenging activity (%) at different concentrations (6.25, 12.50, 25.00, 50.00, 100.00, and 200.00 µg/mL), in accordance with previous literature [1,17,23]. The percentage of RSA has been summarised in **Figure 5**.

The IC_{50} values were calculated by plotting the percentage of DPPH RSA versus concentration. The inhibitions of **1a-g** to reduce DPPH concentration by 50% (IC_{50}) are summarised in **Figure 6**. Antioxidant analysis revealed that the synthesised compounds (**1a–g**) exhibited IC_{50} values ranging from 116.26 to 268.61 µg/mL (equivalent to 386.01 to 1,060.05 µM) (**Figure 6**). In addition to the phenolic –OH, the imine moiety (C=N) is believed to enhance antioxidant effects via HAT and SET mechanisms [18]. The extended π-system can stabilise the radical formed upon hydrogen donation, thereby improving radical-scavenging ability. Among the synthesised derivatives, compound **1f** was identified as the most potent antioxidant. Although compound **1c** demonstrated a lower IC_{50} based on mass concentrations (116.26 µg/mL), compound **1f** exhibited better molar concentration with an IC_{50} of 386.01µM (compared to 444.23 µM for **1c**). This phenomenon indicates that compound **1f** has a higher intrinsic efficiency per molecule for radical scavenging. Halogen as a substituent has been reported to exhibit better biological activity due to its larger size and greater polarizability for effective delocalisation and stabilisation of the unpaired electron with the intermediate radical [24,33]. The halogen substituent at the para position is also often reported to be favourable for biological interactions [34].

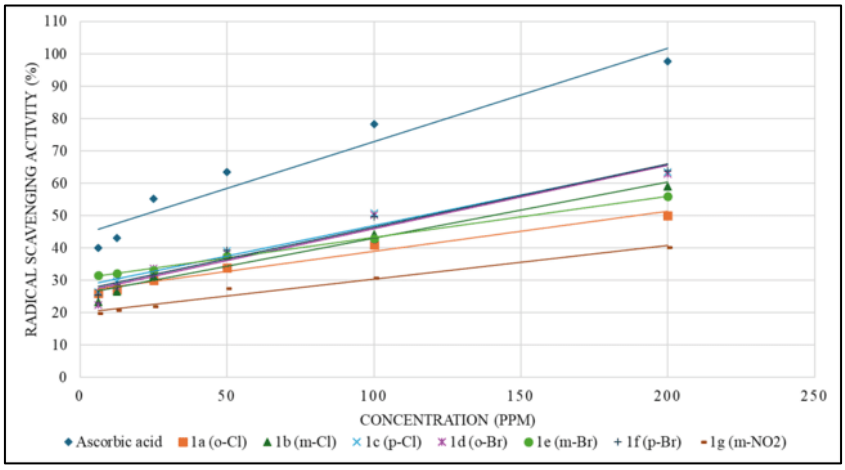


Figure 5. Percentage of radical-scavenging activity of vanillin Schiff bases **1a–g** against DPPH radicals

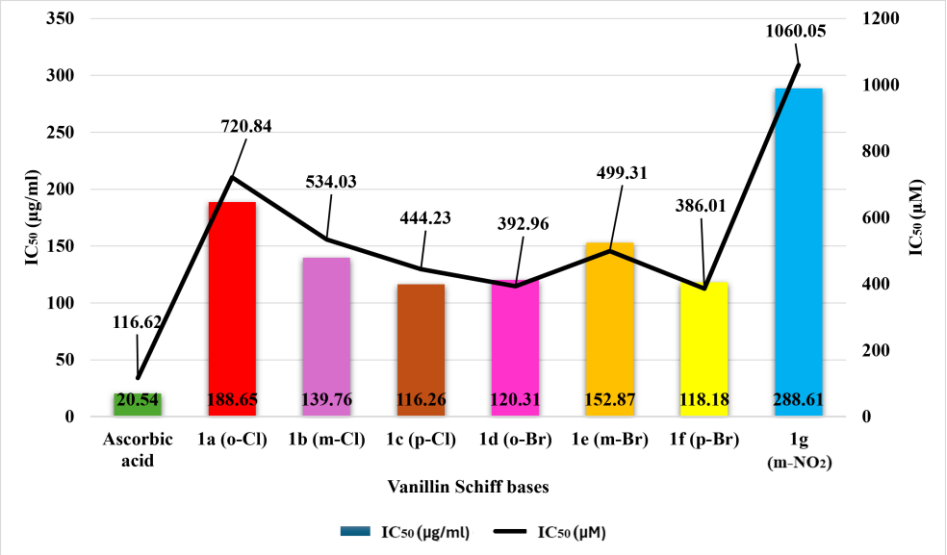


Figure 6. IC_{50} values of vanillin Schiff bases **1a-g**

The IC_{50} of **1a-g** displays a correlation between substituents and their radical-scavenging potency. Comparing the EWGs of halogen and NO_2 groups, halogen contributes better antioxidant effects than NO_2. Halogens can enhance antioxidant activity more effectively than nitro groups due to their ability to stabilise the resulting radical via resonance effects, counteracting their inductive electron-withdrawing nature [35]. This finding suggests a balanced electronic state in which the inductive withdrawal is partially compensated by resonance stabilisation of the radical intermediate. However, reducing the electron density across the conjugated imine (C=N) system can increase the oxidation potential and destabilise radical intermediates due to the strong electron-withdrawing capacity of the NO_2 group, thereby inhibiting the molecule's ability to neutralise free radicals [36]. The incorporation of the imine moiety, particularly when combined with functional groups such as halogens (Cl, Br), significantly enhances free radical scavenging [5], thereby improving antioxidant capacity relative to vanillin alone. This finding highlights that the imine bridge (C=N) effectively transmits substituent electronic effects throughout the molecular scaffold, thus influencing their antioxidant properties.

***In silico* molecular docking analysis**

Computational analysis was also carried out to understand the antioxidant mechanism of compounds with moderate activities (**1a-f**) via molecular docking analysis using AutoDock Tools and AutoDock Vina [26]. Compounds **1a–f,** which exhibited moderate IC_{50} values, were evaluated for their mechanism of action in inhibiting human 5-LOX (PDB ID: 6N2W) [22]. Human 5-lipoxygenase is a non-heme iron-containing enzyme that initiates the biosynthesis of pro-inflammatory leukotrienes. It can drive oxidative stress by catalysing the oxidation of polyunsaturated fatty acids into lipid hydroperoxides, which are highly reactive and contribute to cellular damage [37]. The enzyme activity of 5-LOX is a major source of ROS that can damage cells and contribute to various inflammatory and chronic diseases. Vanillin Schiff bases can possibly inhibit 5-LOX, which suggests their potential as antioxidant agents. Prior to the in silico molecular docking of compounds **1a–f**, docking validation was performed. Upon re-docking, the extracted NDGA from the original crystal structure's binding site were superimposed on the co-crystallised NDGA inhibitor, yielding an RMSD of 2.005 Å. The RMSD analysis was performed by aligning each re-docked pose to the reference structure, thereby evaluating the structural similarity between the generated pose and the co-crystallised NDGA inhibitor conformation (**Figure 7**). An RMSD value less than 3.00 Å is generally considered good for the accuracy of docking predictions [38].

Docking parameters for validation were replicated to evaluate the biological interactions of **1a–f** yielding different binding energies (-6.30 to -6.00 kcal/mol) (**Table 1**). These binding energy values are comparable to those of standard inhibitor NDGA (6.60 kcal/mol), a known 5-LOX inhibitor [39], which is also widely utilised as an antioxidant agent in pharmaceutical formulations and cosmetic products [40]. The visual interpretation of receptor–ligand complex structures for **1a–f** and NDGA is available in the supplementary files (**Table S1).** The docking analysis of **1a-f** showed that good binding affinities were also consistent with the *in vitro* DPPH assay.

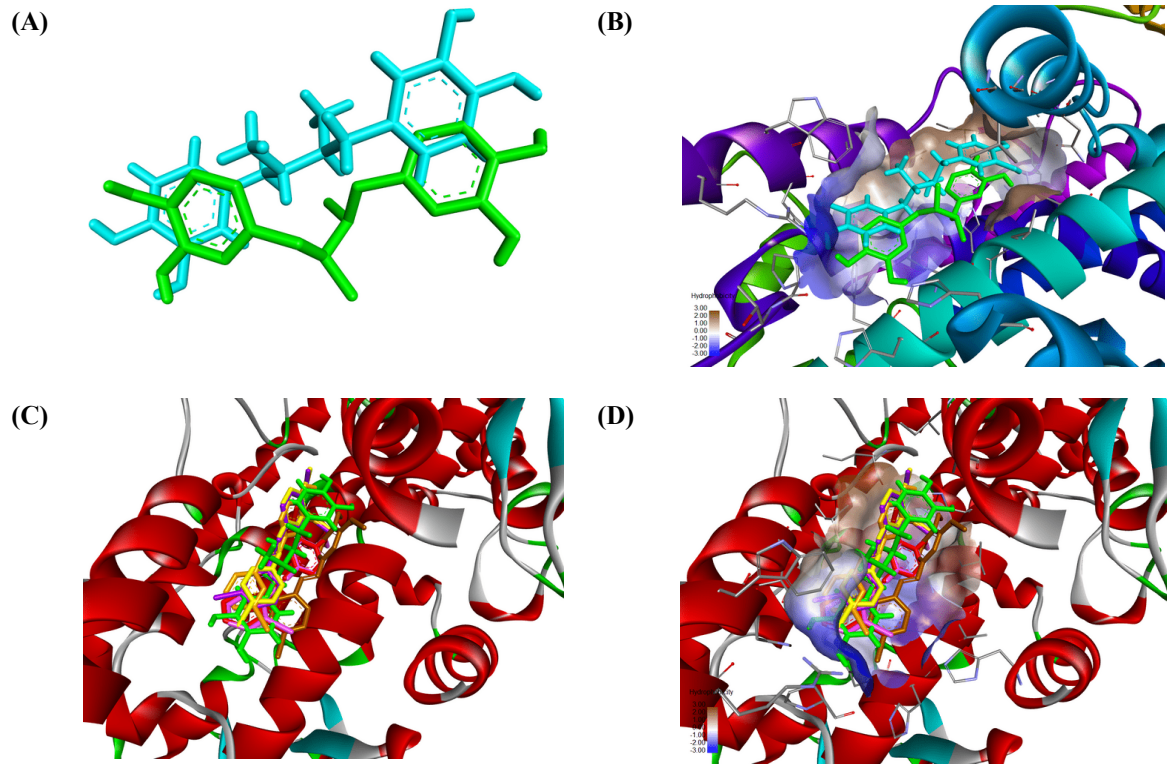


Figure 7. Validation of docking parameters for replication of output using stick model as ligand. **A**: Superimposition of re-docked (cyan) & co-crystallised NDGA (green) inhibitors, with an RMSD of 2.005 Å. **B**: Orientation of re-docked inhibitors (cyan) and co-crystallised inhibitors (green) within the DNA gyrase binding site. **C**: Orientation of the co-crystallised inhibitor (green), **1a** (red), **1b** (purple), **1c** (brown), **1d** (pink), **1e** (orange), and **1f** (yellow) within the binding site of 5-LOX. **D**: Alignment of the co-crystallised inhibitor (blue), **1a** (red), **1b** (purple), **1c** (brown), **1d** (pink), **1e** (orange), and **1f** (yellow) in the hydrophobic pocket of 5-LOX

Table 1. Binding scores of synthesised compounds **1a–f** compared with the reference compound NDGA

Compounds	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	NDGA
Binding score (kcal/mol)	-6.00	-6.10	-6.20	-6.30	-6.10	-6.20	-6.60

Representing vanillin Schiff bases, compound **1f** exhibited a favourable binding interaction with the target receptor (-6.30 kcal/mol), primarily through hydrogen bond interactions between OH and OCH3 with ARG596 and PRO569 residues. The OCH3 of vanillin also formed binding interactions with the amino acid residues of PHE359, PRO569, TRP599, and HIS600 (alkyl and π-alkyl interactions). Similar alkyl and π-alkyl interactions also formed between compound **1f** and the HIS372 and LEU368 residues. Compound **1f** also formed π-π-T-shaped interactions with the amino acid of PHE359 through the aromatic ring of vanillin, further enhancing its antioxidant properties. Additionally, the lipophilic properties of **1a–f** within the hydrophobic pocket of the 5-LOX active site contributed to the favourable binding interactions [41,42] as illustrated in **Figure 7**.

Pharmacokinetic studies

The drug-likeness and ADMET profiles of vanillin Schiff bases (**1a–g**) were further evaluated through online software tools, SwissADME and pkCSM. This computational study was conducted using Lipinski's Rule of Five (Ro5), thereby providing an initial understanding of their potential pharmacokinetic properties. All compounds showed no violations against Lipinski's Ro5 (**Table 2**). An ideal drug candidate typically meets the criteria of a hydrogen bond donor and acceptor of less than 5 and 10, respectively, MW below 500 Da, a log *P* value under 5, and a a topological polar surface area (TPSA) less than 140 Å [1,43].

Table 2. Drug-likeliness predictions of **1a–g** by SwissADME

Compound	Molecular Formula	MW (g/mol)	NHD	NHA	NRB	TPSA	Log *P* (iLOGP) Lipophilicity	Log *P* (MLOGP) Lipophilicity	Log *S* (ESOL) Water Solubility	Lipinski's Ro5 Violations
1a	$C_{14}H_{12}ClNO_2$	261.71	1	3	3	41.82	2.89	2.78	-4.08	0
1b	$C_{14}H_{12}ClNO_2$	261.71	1	3	3	41.82	2.98	2.78	-4.08	0
1c	$C_{14}H_{12}ClNO_2$	261.71	1	3	3	41.82	2.99	2.78	-4.08	0
1d	$C_{14}H_{12}BrNO_2$	306.16	1	3	3	41.82	3.01	2.91	-4.39	0
1e	$C_{14}H_{12}BrNO_2$	306.16	1	3	3	41.82	3.08	2.91	-4.39	0
1f	$C_{14}H_{12}BrNO_2$	306.16	1	3	3	41.82	3.02	2.91	-4.39	0
1g	$C_{14}H_{12}N_2O_4$	272.26	1	5	4	87.64	2.26	1.24	0.082	0

Table 3. ADMET predictions of compounds **1a–f** computed using SwissADME and pkCSM

Compound	GI Absorption	BBB Permeant	Cytochrome P450 (CYP) Substrate			Cytochrome P450 (CYP) Inhibitor				Clearance	hERG I & II Inhibitor	Hepatoxicity
			2D6	3A4	1A2	2C19	2C9	2D6	3A4			
1a	High	Yes	Yes	Yes	Yes	Yes	Yes	No	No	0.280	No	No
1b	High	Yes	Yes	Yes	Yes	Yes	No	No	No	0.155	No	No
1c	High	Yes	Yes	Yes	Yes	Yes	Yes	No	No	0.092	No	No
1d	High	Yes	Yes	Yes	Yes	Yes	Yes	No	No	0.044	No	No
1e	High	Yes	Yes	Yes	Yes	Yes	Yes	No	No	0.133	No	No
1f	High	Yes	Yes	Yes	Yes	Yes	Yes	No	No	0.071	No	No
1g	High	No	No	Yes	Yes	Yes	Yes	No	Yes	0.109	No	No
Software Used	SwissADME	SwissADME	pkCSM							pkCSM	pkCSM	pkCSM

The ADMET profiles of **1a-g** were further predicted via SwissADME and pkCSM (Table 3). All synthesised compounds are potentially suitable for oral administration, exhibiting high GI absorption, while BBB permeability was observed only for compounds **1a–f** [44]. Compounds exhibiting excellent GI and BBB permeation are highly desirable for therapeutic agents targeting the central nervous system, as they ensure effective oral absorption and brain accessibility [45,46]. Prediction for metabolism activity revealed that nearly all compounds are substrates for CYP2D6 and CYP3A4. This analysis suggests that the compounds undertake metabolic activity through major cytochrome P450 pathways [47]. In addition, most of the synthesised compounds can partly affect the metabolic stability through the inhibition of CYP1A2, CYP2C19, and CYP2C9 [48]. CYP inhibitors or inducers possibly affect the pharmacokinetics, which underscores the importance of optimising future therapeutic agents.

The predicted clearance rates for the synthesised compounds were low, which indicates prolonged systemic retention and enhanced bioavailability [43]. The synthesised compounds demonstrated a favourable safety profile, with no predicted hERG inhibition, thereby minimising the risk of cardiac toxicity [47]. Compounds **1a–g** also showed no predicted hepatotoxicity, reducing the likelihood of liver damage [49]. The BBB permeability of the compound series was evaluated using the SwissADME BOILED-Egg model, which predicts absorption based on the correlation between lipophilicity (WLOGP) and polarity (TPSA) as presented in Table 2. Compounds **1a-f** are positioned within the yellow region, indicating high probability for GI absorption and BBB penetration (Figure 8). However, compound **1g** is located in the white region, which indicates high absorption but limited BBB permeability. All compounds are less likely to undergo active efflux via P-glycoprotein, as these compounds were represented by red circles (PGP-).

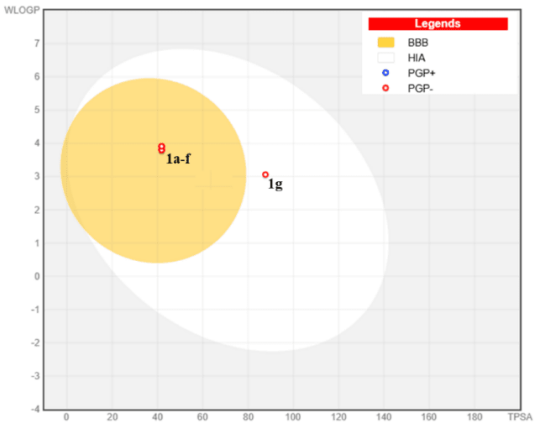


Figure 8. BOILED-Egg of vanillin Schiff bases (**1a–g**)

Conclusion

In conclusion, hybrid Schiff bases derived from vanillin (**1a–g**) were successfully synthesised and evaluated for their antioxidant potential. Most of the synthesised compounds (**1a–f**) exhibited notable antioxidant activity in the DPPH assay with moderate IC_{50} values. Compound **1f** showed the best antioxidant activity among the synthesised compounds. Docking analysis supported its potential as an antioxidant agent due to the binding affinity of -6.30 kcal/mol and comparable to that of the standard inhibitor NDGA (-6.60 kcal/mol). This finding suggests that compound **1f** demonstrates good interactions with the target receptor with good ADMET properties. These preliminary results of using DPPH assay highlight the importance of Schiff bases derived from vanillin as a strategic approach to combat diseases related to oxidative stress. Therefore, future research will include additional antioxidant methods (ORAC, FRAP, ABTS) to explore these compounds as potential antioxidant agents.

Acknowledgement

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