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MICROWAVE-ASSISTED SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ZINC(II) SCHIFF BASES FOR DPPH RADICAL SCAVENGING ACTIVITY

G. Subash[1,✉] and M. Senthil raja[1]
[1]Department of Pharmaceutical Chemistry, P.S.V. College of Pharmaceutical Science and Research, Krishnagiri-635108, Tamil Nadu, India.
Corresponding author: kuttysubash1127@gmail.com

ABSTRACT
Molecular docking was conducted on a set of twenty compounds (SB1 to SB20) targeting the Kelch domain of keap1 (PDB: 8A46). Among the docked compounds, SB1 to SB5 showed strong binding affinity, whereas SB6 to SB20 exhibited moderate affinity. A series of five novel bis-phenolate Zn(II) Schiff bases (SB1-SB5) were synthesised by condensing vanillin with structurally diverse aromatic amines, followed by complexation with Zn(II) using microwave irradiation. Compounds were analysed using spectroscopic studies and LC-HRMS. Antioxidant potential was screened by the DPPH assay at 5–320 µg/mL, resulting in IC50 values between 13.85 and 26.70 µg/mL. Notably, compounds SB1, SB2 and SB4 demonstrated strong free radical scavenging capabilities, whereas SB3 and SB5 exhibited moderate activity in comparison to the reference standard, ascorbic acid.
Keywords: Zn(II) Schiff Bases, Spectroscopy, AutoDock4, Molecular Docking, DPPH Antioxidant
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INTRODUCTION

Schiff bases, which are formed by condensing primary amines with aldehydes or ketones, have attracted widespread attention as potential therapeutic agents. Their imine (–C=N–) linkage is chemically versatile and readily tuned through variation of the amine and carbonyl precursors, allowing control over lipophilicity, redox behaviour, and metal-binding affinity1. This structural adaptability, combined with their well-documented antioxidant, anti-inflammatory, and metal-chelating properties, has positioned Schiff bases as attractive scaffolds for multi-target-directed ligand design, an approach increasingly favoured for multifactorial diseases for which a single-target drug is unlikely to be effective.[1] A common observation in the literature is that when Schiff base ligands form complexes with metal ions, notably zinc(II), copper(II), and other transition and post-transition metals, their biological activity is generally increased rather than decreased. Zinc, an ion crucial for physiological processes and known for not participating in redox reactions, is well-tolerated in living organisms[2]. Schiff base demonstrated potent free-radical scavenging in DPPH, superoxide, and hydroxyl radical assays and produced measurable neuroprotective and antihyperglycemic effects in cerebral ischemia and streptozotocin-induced diabetic mouse models.[3] Similarly, zinc carboxylate complexes that include amide linkages and additional heterocyclic ligands have demonstrated significant antioxidant properties in DPPH assays, with molecular docking revealing stable interactions at the active sites of acetylcholinesterase.[4] Collectively, these findings support the view that metal incorporation into a Schiff base scaffold can improve radical-scavenging capacity, membrane permeability, and target engagement relative to the free ligand.[5] Oxidative stress arises from an imbalance between the generation of the body's antioxidant response and reactive oxygen species (ROS) and is now acknowledged as a significant factor for chronic and degenerative diseases, such as cerebral ischemia, diabetes mellitus, and Alzheimer's disease (AD).[6] An overproduction of ROS leads to damage in lipids, amino acids and nucleic acids, with the central nervous system being affected due to the brain's high oxygen consumption and relatively low natural antioxidant capacity.[7] Consequently, there is sustained interest in identifying compounds, whether of natural or synthetic origin, capable of scavenging free radicals and mitigating oxidative damage. Oxidative stress and both oxidative stress and MAO-B-mediated dopamine catabolism are implicated in neurodegenerative pathology, and MAO-B inhibitors are used clinically to limit oxidative dopamine

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metabolism. Simultaneously, activation of the Keap1–Nrf2 pathway enhanced the body's antioxidant defences, offering another neuroprotective strategy by mitigating neuronal damage caused by ROS.[7] Schiff base derivatives, which may influence Keap1–Nrf2 signalling, chelate metal ions involved in amyloid-β aggregation, and directly neutralise free radicals, have therefore been suggested as promising multi-target neuroprotective candidates.[8] A progressed Schiff base as far as early-phase clinical trials for Alzheimer's disease before its development was discontinued, underscoring both the therapeutic promise and the translational challenges associated with this compound class.[8] This research builds on earlier studies by synthesising vanillin-derived Zn(II) Schiff base complexes using a microwave-assisted method. These complexes were characterised through various spectroscopic techniques, and their antioxidant properties were evaluated using the DPPH radical scavenging assay, along with molecular docking studies involving the Kelch domain of Keap1. The aim was to determine if the improved radical-scavenging and metal-chelating properties observed in related Schiff base–metal complexes could be replicated in a straightforward, synthetically accessible vanillin–Zn(II) system and to explore its potential as a multi-target antioxidant.

Research Gap: Vanillin-based Zn(II) Schiff bases made by microwave synthesis haven't been studied for combined DPPH antioxidant activity and Keap1 (Nrf2 pathway) docking as multi-target antioxidants.

EXPERIMENTAL

Materials and Methods

IR spectra were recorded on an IR Affinity-1/Thermo Nicolet iS50 instrument fitted with an ATR attachment. ^{1}H NMR spectra were measured at 400 MHz on a Bruker Avance III spectrometer, with chemical shifts given in δ (ppm) relative to tetramethylsilane (TMS) as internal standard. Mass spectra were collected by LC-HRMS (positive and negative ion modes) on a XEVO-G2-XS-QTOF instrument. DPPH absorbance readings were taken on a Shimadzu UV-1900i UV-Vis spectrophotometer. Reactions were monitored by TLC on pre-coated Merck silica gel plates developed in ethyl acetate: petroleum ether (1:4) and visualised with iodine. The structures of the products were confirmed from IR, NMR and mass spectral data. Chemicals and reagents from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt. Ltd. (India) were used as received, without further purification.

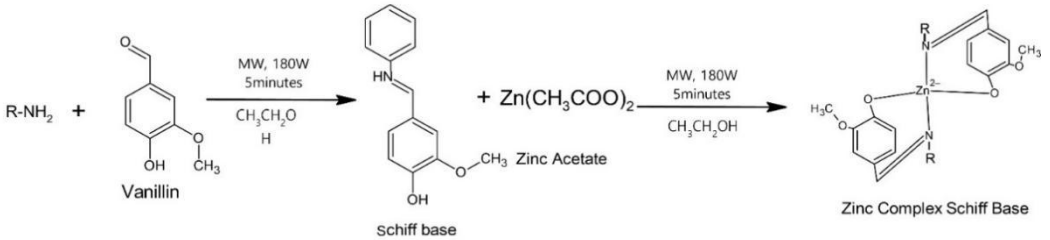
General Procedure

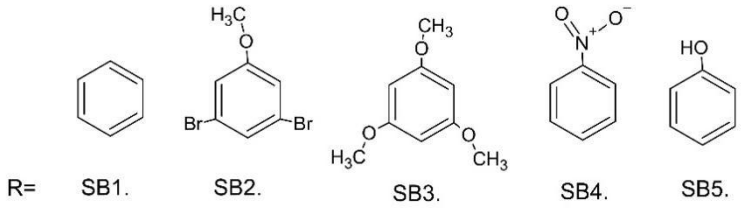
Synthesis of Schiff Base

Weigh accurately about 3.35 g 0.02 mol) of vanillin and dissolve it in 5mL of ethanol in a clean conical flask and add 2 mL or 2 g (0.02 mol) of various anilines [(aniline), (2,6-dibromo-4-methoxy aniline), (2,4,6 tri methoxy aniline), (4-nitro aniline), (4-aminophenol)] and mix. The resulting mixture was placed in a microwave oven at 180 W for 5 minutes; the yellow mixture turned red. After allowing the mixture to reach room temperature, it was submerged in an ice bath. The precipitate was filtered and recrystallised using ethanol.

Synthesis of Zinc (II) Schiff Base Complex

Weigh accurately about 4.35 g (0.02 mol) of Schiff base dissolved in 30mL of ethanol in a clean conical flask. In a separate clean beaker, precisely measure approximately 2.1 g (0.01 mol) of zinc acetate and dissolve it in 20 mL of ethanol. This solution was gradually introduced to the Schiff base solution and thoroughly mixed. The mixture was then subjected to microwave heating at 180W for 5 minutes, transforming the yellowish mixture into a red solution. After cooling to room temperature, it was submerged in an ice bath. The formation of precipitate was filter and recrystallised using ethanol.





Scheme-1: Synthesis of Zinc(II) Schiff Base

Analytical and Computational Methods

Spectral Characterization

FTIR spectra were acquired by ATR over the range 4000–400 cm^{-1}. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$, with chemical shifts (δ, ppm) referenced to TMS. Molecular ions were confirmed by LC-HRMS in ES+ mode.

Molecular Docking Studies

Docking was performed using AutoDock 4 against the protein targets, focusing on the Kelch domain of Keap1 (PDB: 8A46), selected to evaluate its potential influence on the Keap1–Nrf2 pathway, crucial for the body's antioxidant response. Grid and docking parameter files were prepared using AutoDock Tools; the optimised 3D structures of compounds (SB1–SB5) were docked into the respective binding sites, and the pose with the lowest (most negative) estimated free energy of binding was selected for each compound.

DPPH Radical Scavenging Assay

The antioxidant activity of SB1–SB5 were determined by the DPPH free radical scavenging assay. DPPH was dissolved in methanol to give a 0.1 mM stock solution. Test samples were prepared across a 5–320 µg/mL concentration range and mixed with DPPH solution against ascorbic acid as the reference standard. The mixtures were incubated in the dark at room temperature for 30 min, and absorbance was then read at 517 nm against a DPPH-only control. Percentage inhibition was calculated using:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100 \quad (1)$$

IC_{50} values, which indicate the concentration needed for 50% radical scavenging, were calculated through linear interpolation between the concentrations surrounding 50% inhibition for each compound and ascorbic acid.

RESULTS AND DISCUSSION

SB1-Bis{4-methoxy-2-[(E)-(phenylimino)methyl]phenolato-κO,κN}zinc(II)

Yield 78%. m.p. 206°–210°C. IR (ATR, cm^{-1}): 3059 (Ar C–H), 1581 (C=N), 1512, 1458, 1427 (C=C), 1358 (C–O), 1280–1022 (C–O–C), 810, 764, 690 (mono-sub. N-phenyl), 613, 548, 486, 440 (Zn–O/Zn–N). ^{1}H NMR ($CDCl_3$) δ (ppm): 8.37 (s, 2H, HC=N), 7.66–6.70 (m, 14H, Ar-H), 4.01 & 3.98 (s, 6H, 2×OCH_3). ^{13}C NMR ($CDCl_3$) δ (ppm): 160.2 (HC=N), 151.7, 147.2, 146.3 (Ar–C), 129.9–108.8 (Ar–CH), 56.2, 56.1 (OCH_3). LC–HRMS (m/z): 516.39 [M+H]+ (calcd. 517.11 for $C_{28}H_{25}N_2O_4Zn$).

SB2-Bis{2-[(E)-(2,6-dibromo-4-methoxyphenylimino)methyl]-4-methoxyphenolato-κO,κN}zinc(II)

Yield 73%. m.p. 255°–260°C. IR (ATR, cm^{-1}): 3410, 3287, 3071 (br, moisture/EtOH), 1663 (C=N), 1605, 1539 (C=C), 1258–1026 (Ar–OCH_3/C–O–C), 853 (isolated Ar-H), 725, 702 (C–Br), 660–428 (Zn–O/Zn–N). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.53–7.06 (m, Ar-H), 4.59 (s, Ar-OCH3), 3.99 (s, Ar-OCH3). ^{13}C NMR ($CDCl_3$) δ (ppm): 151.7 (HC=N), 141.3 (C–N), 133.8 (C–Br), 127.5, 114.4, 108.8 (Ar-CH), 56.2 (OCH_3). LC–HRMS (m/z): 896.65 [M+H]+ (calcd. 888.77 for $C_{30}H_{25}Br_4N_2O_6Zn$; isotope cluster 886–912).

SB3-Bis{2-[(E)-(2,4,6-trimethoxyphenylimino)methyl]-4-methoxyphenolato-κO,κN}zinc(II)

Yield 67%. m.p. 210°–216°C. IR (ATR, cm^{-1}): 3410, 3287, 3071 (moisture/EtOH), 2920, 2851 (sp^3 C–H), 1667 (C=N), 1609, 1539 (C=C), 1265–1018 (Ar–OCH3), 853, 725, 702 (Ar-H), 629–417 (Zn–O/Zn–N). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.53–6.22 (m, Ar-H), 4.01/3.99 (s, Ar-OCH3, overlapping). ^{13}C NMR ($CDCl_3$) δ (ppm): 151.7 (HC=N), 147.1 (C–OMe), 141.3, 133.8, 129.9, 127.6 (Ar-C), 114.4, 108.8 (Ar-CH), 56.2 (OCH_3). LC–HRMS (m/z): 699.48 [M+H]+ (calcd. 697.17 for $C_{34}H_{37}N_2O_{10}Zn$).

SB4-Bis{4-methoxy-2-[(E)-(4-nitrophenylimino)methyl]phenolato-κO,κN}zinc(II)
Yield 82%. m.p. 250°–253°C. IR (ATR, cm^{-1}): 3476, 3352 (Ar C–H), 1628 (C=N), 1585 (νasym NO_2), 1466, 1443 (C=C), 1319 (νsym NO_2), 1177, 1111 (C–O), 995, 899, 837 (ring C–H), 748, 694, 629 (C–H), 532, 486, 417 (Zn–O/Zn–N). ^{1}H NMR ($CDCl_3$) δ (ppm): 8.23–8.07 (m, Ar-H ortho to NO_2), 7.28–6.63 (m, Ar-H), 4.41 (s, OCH_3). ^{13}C NMR ($CDCl_3$) δ (ppm): 152.4 (HC=N/C–O), 139.2 (C–NO_2), 126.4, 126.0, 125.4, 125.3 (Ar-CH), 113.4 (Ar-CH), 56.2 (OCH_3). LC–HRMS (m/z): 607.42 [M+H]+ (calcd. 607.08 for $C_{28}H_{23}N_4O_8Zn$).

SB5-Bis{2-[(E)-(4-hydroxyphenylimino)methyl]-4-methoxyphenolato-κO,κN}zinc(II)
Yield 71%. m.p. 264°–270 °C. IR (ATR, cm^{-1}): 3267 (OH, free), 1585 (C=N), 1508, 1427 (C=C), 1369 (C–O), 1281, 1242, 1204 (Ar–OCH_3), 1150, 1123, 1026 (C–O–C), 968, 860, 833, 783, 733 (Ar-H), 610, 532, 501, 463 (Zn–O/Zn–N). ^{1}H NMR ($CDCl_3$) δ (ppm): 9.85 (br s, free OH), 7.46–6.22 (m, Ar-H), 4.01/3.96 (s, OCH3). ^{13}C NMR ($CDCl_3$) δ (ppm): 151.7 (HC=N), 147.1 (C–OH), 129.9, 127.6, 114.4, 108.7 (Ar-CH), 56.1 (OCH_3). LC–HRMS (m/z): 549.38 [M+H]+ (calcd. 549.10 for $C_{28}H_{25}N_2O_6Zn$). The spectral data (FTIR, ^{1}H/^{13}C NMR, LC–HRMS) confirm that compounds (SB1–SB5) are the symmetric Zn(II) Schiff base compounds. The loss of the aldehyde and phenolic OH signals, the appearance of a new C=N stretch and Zn–O/Zn–N bands, confirm this for all five compounds. The melting points for the series ranged from 206° to 270°C; compound SB5, featuring a free phenolic OH group, exhibited the highest melting point due to additional hydrogen bonding, whereas compound SB1 had the lowest.

Molecular Docking Studies
All five compounds exhibited strong predicted binding affinities to the Keap1 Kelch domain, with docking scores ranging from −8.44 to −10.36 kcal/mol. Compound SB2 (2,6-dibromo-4-methoxyphenyl-substituted) showed the highest affinity (−10.36 kcal/mol), followed by compound SB4 (nitro-substituted, −9.62 kcal/mol), suggesting that the electron-withdrawing/halogenated substituents may enhance engagement with key residues in the Kelch β-propeller binding pocket. Compound SB1 (−9.38 kcal/mol) and compound SB5 (−9.01 kcal/mol) also showed favourable binding, while compound SB3 (trimethoxy-substituted) showed the comparatively weakest, though still substantial, affinity (−8.44 kcal/mol). Overall, these findings suggest that Zn(II) Schiff base compounds may exert their effects by interfering with the Keap1–Nrf2 interaction, which in turn promotes Nrf2 stabilisation, nuclear translocation and the upregulation of downstream antioxidant response element (ARE)-driven gene expression, offering a plausible complementary mechanism to any direct radical-scavenging activity.

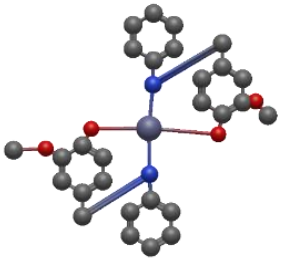
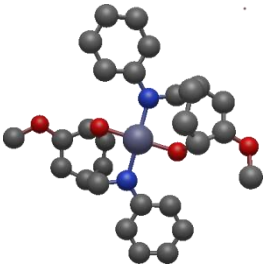
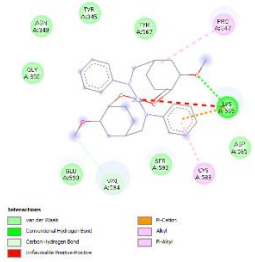
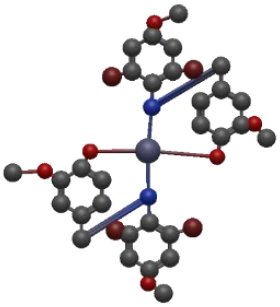
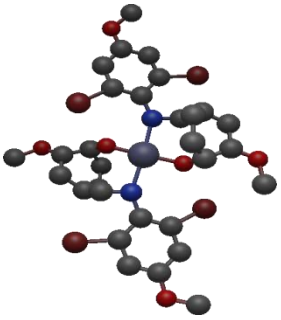
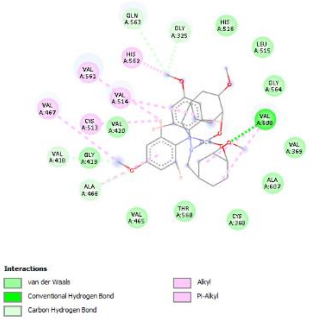
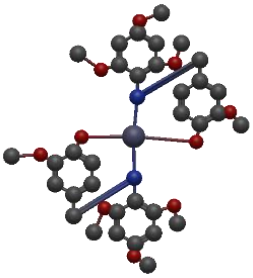
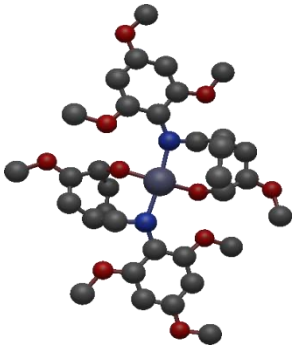
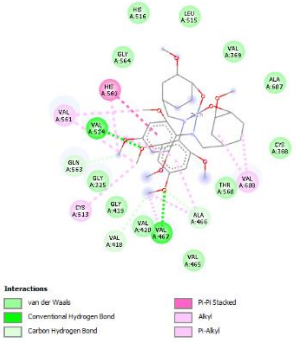
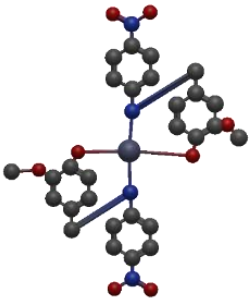
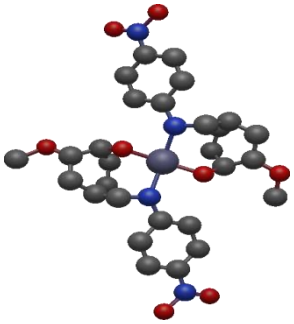
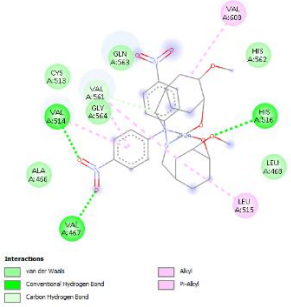
Table-1: Binding Energies of Compounds (SB1–SB20) to the Target Protein 8A46

Code	Binding energy (kcal/mol)	Code	Binding energy (kcal/mol)	Code	Binding energy (kcal/mol)	Code	Binding energy (kcal/mol)
SB1	−9.38	SB6	−6.97	SB11	−8.32	SB16	−7.91
SB2	−10.36	SB7	−7.81	SB12	−8.41	SB17	−8.23
SB3	−8.44	SB8	−7.15	SB13	−7.64	SB18	−7.90
SB4	−9.62	SB9	−6.77	SB14	−7.54	SB19	−8.03
SB5	−9.01	SB10	−7.02	SB15	−8.11	SB20	−7.21

Structure optimisation before docking was essential to prevent the ligand from breaking into multiple pieces. Structure optimisation was performed using Avogadro2. In the docking study against the Keap1 protein, all five compounds showed good binding, with scores between −8.44 and −10.36 kcal/mol. Compound SB2 bound the strongest, followed by SB4. This suggests that groups that pull electrons away from the ring help the compound fit and bind better into the protein's pocket. Compound SB3, which has three methoxy groups and is the bulkiest molecule, bound the weakest, showing that a compound's size and shape matter more for binding than simply having more electron-donating groups.

Antioxidant Activity (DPPH assay)
The radical scavenging capacity of SB1–SB5 was measured by the DPPH assay across a 5–320 μg/mL dose–response range (Control absorbance = 0.840 at 517 nm), as a preliminary indicator of antioxidant activity. Percentage inhibition at each tested concentration was summarised in Table-3.

Table-2: 3D Structure, 3D Optimized Structure and 2D Interactions of Compound (SB1–SB5) to the Targeted Protein 8A46

Code	3D Structure	Optimized 3D structure	2D INTERACTION OF 8A46
SB1.			
SB2.			
SB3.			
SB4.			

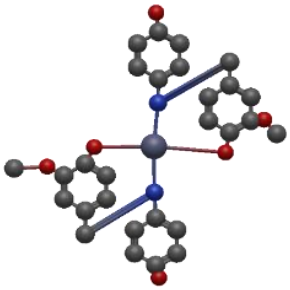
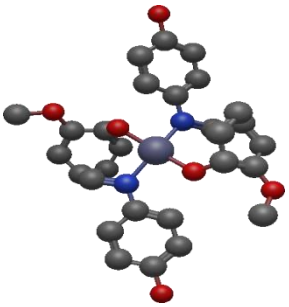
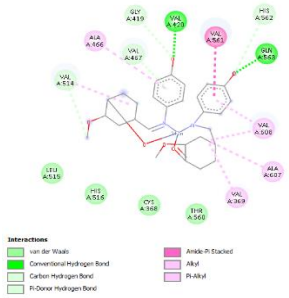
SB5.			

Table-3: DPPH Percentage Inhibition (%) Across the Concentration Range 5–320 µg/mL (concentrations in µg/mL)

Compound	5 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	160 µg/mL	320 µg/mL
SB1	16.19	38.65	68.14	90.43	97.29	98.17	98.57
SB2	16.14	38.57	67.00	79.71	92.00	94.14	95.71
SB3	5.00	21.00	45.43	74.00	90.71	93.00	93.94
SB4	12.00	32.14	59.71	73.00	88.14	91.29	95.62
SB5	2.86	17.43	40.43	69.00	91.57	92.71	93.14
Ascorbic acid	10.03	20.88	48.71	89.86	95.43	96.43	97.43

Table-4 shows an overview of IC_{50} values that were calculated by linear interpolation between the doses spanning 50% inhibition.

Table-4: DPPH IC_{50}Values (linear interpolation)

Compound	IC_{50} (µg/mL)
SB1	13.85
SB2	14.02
SB3	23.20
SB4	16.48
SB5	26.70
Ascorbic acid (standard)	20.63

Compounds SB1, SB2 and SB4 showed lower IC_{50} (i.e., greater potency) than the ascorbic acid standard, while compounds SB3 and SB5 were less potent than ascorbic acid. The overall potency order was: compound SB1 (13.85 µg/mL) > compound SB2 (14.02 µg/mL) > compound SB4 (16.48 µg/mL) > ascorbic acid (20.63 µg/mL) > compound SB3 (23.20 µg/mL) > compound SB5 (26.70 µg/mL), with compound SB1 now the single most potent scavenger in the series rather than tying with compound SB2.

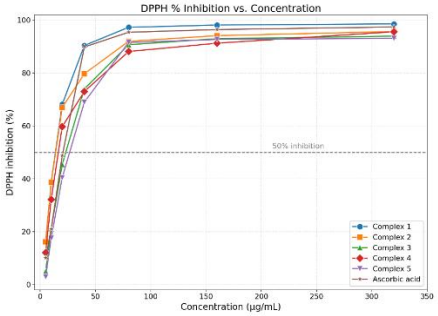


Fig.-1: DPPH Percentage Inhibition (%) vs. Concentration (µg/mL) for SB1–SB5 and Ascorbic Acid

The ascorbic acid IC_{50} falls within a concentration between (20–40 µg/mL) have been calculated, all IC_{50} values reported here were obtained by linear interpolation. For antioxidant activity, compounds SB1, SB2 and SB4 show free radical scavenging more effectively than the ascorbic acid standard, while compounds SB3 and SB5 were weaker. Compound SB1 was the single most potent scavenger, even though its Keap1 binding was only moderate; compound SB2 was the strongest binder, ranked second in scavenging;

compound SB4 ranked third in scavenging; and compound SB4 and SB5 showed moderate free radical scavenging.

CONCLUSION

In this study, twenty compounds [SB1-SB20] were utilised for molecular docking studies using Keap I-Kelch domain (protein ID: 8A46). Among the docked compounds, only five compounds showed prominent binding affinity to the selected protein (8A46) model, and the same compounds were synthesised using a fast, microwave method. The characterisation of all synthesised compounds was conducted using TLC, chemical tests and spectral data, followed by purification with ethanol. All the compounds (SB1 to SB5) were screened for antioxidant activity using the DPPH method having 5-320µg/mL concentration respectively. Among the screened compounds, compound (SB1) shows prominent free radical inhibition activity; other compounds show moderate free radical inhibition activity, and the IC_{50} values show that compound (SB1) has a lower concentration to achieve 50% antioxidant activity; other compounds show comparatively higher concentrations to achieve 50% antioxidant activity. Therefore, compound (SB1) shows prominent antioxidant activity.

ACKNOWLEDGMENTS

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

G. Subash https://orcid.org/0009-0005-1153-3181
M. Senthil Raja https://orcid.org/0000-0003-2005-4420

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