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RESEARCH ARTICLE

ANTIOXIDANT DENDRIMERS WITH SCHIFF'S BASE: SYNTHESIS AND *IN-VITRO* ANALYSIS

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ABSTRACT

Dendrimers with anthranilate derived Schiff's base as surface unit were synthesized by divergent approach and their absorption, emission and anti-oxidant properties were carried out. The absorption, emission and anti-oxidant property increases with increase in the generation of dendrimers and the anti-oxidant behaviour shows to be dose dependent.

INTRODUCTION

As fluorescence property has tremendous applications in molecular biology (Zhang *et al.*, 2002; Sailh *et al.*, 2000; Chalfie, 1995; Chalfie *et al.*, 1994), synthesizing such compounds evokes its importance among researchers. Fluorescence-labeled compounds have opened a wide range of applications in PCR, DNA sequencing, microarrays. Hence, attracted by such reports, cyclophanes and dendrimers with fluorescent property has been reported from our research group, recently (Rajakumar, 2004; Rajakumar, 2005; Rajakumar, 2012). Methyl anthranilate, principal flavorant of Concord grape beverage products, belongs to an interesting class of UV absorbers (Shaath, 1987; Shaath *et al.*, 2005), thus it absorbs UV-A radiation (320-400 nm). Unlike BMDMB (4-tertbutyl-4'methoxydibenzoylmethane, avobenzene, parsol 1789), which is photounstable, Anthranilates are stable (Ozer *et al.*, 2001; The Chemistry of Fragrances) and safe and also listed in GRAS (generally recognized as safe) compounds. Methyl anthranilate is also used to produce Schiff's base with aldehydes, many of which are also used in perfumery (An Introduction to Perfumery by Curtis & Williams 2nd Edition, 2009; Shaath *et al.*, 1990). Owing to its *ortho* effect, anthranilates do not exhibit any significant solvent shifts in cosmetic formulations (Agrapidis-Paloympis *et al.*, 1987; Hawker, 1995). Such, results attracted chemists to synthesize dendrimers (Hawker, 1990) bearing anthranilate derived Schiff's base at the periphery. Herein, we report the synthesis and anti-oxidant activity of Frechet type dendrimers 1-9 (Figure 1) by O-alkylation (Freeman *et al.*, 2000).

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RESULTS AND DISCUSSION

In the present study anthranilic acid was synthesized by reacting phthalimide with sodiumhypochlorite (4 % w/v, Chlorine) followed by the treatment with conc. HCl. Methyl, ethyl and isopropyl anthranilates were obtained by mere esterification of anthranilic acid with methanol, ethanol and isopropanol respectively. The Schiff's bases 13a-c were synthesized by treating the methyl, ethyl and isopropyl anthranilates with 4-hydroxybenzaldehyde in ethanol in the presence of molecular sieves 4 Å in 69 %, 68 % and 63% yield respectively. The Schiff's bases thus obtained were then subjected to O-alkylation with the corresponding bromo compounds to get the required dendrimers by divergent approach.

Zero-generation dendrimer (G_0) 1-3 were synthesized by treating three equivalents of 13a-c with one equivalent of presynthesized tribromo compound 10¹⁹ in dry acetone in presence of K_2CO_3 to give the dendrimers 1, 2 and 3 in 72 %, 71 % and 68 % respectively (Scheme 1). In the ¹H NMR spectrum of dendrimer 1 the ester methyl proton appeared at δ 3.84 and the methylene protons at δ 5.17 and the N=CH-proton at δ 8.27 along with other aromatic protons. In ¹³C NMR spectrum of dendrimer 1 the ester methyl carbon appeared at δ 51.5 the methylene carbon at δ 69.7 and the N=CH- carbon appeared at δ 163.4 and the ester carbonyl at δ 168.6 in addition to other aromatic carbons. The structure of zero-generation dendrimers 2 and 3 was also confirmed from the spectral and analytical data. In a similar manner the first and second generation dendrimers 4-9 were synthesized by treating the presynthesized hexabromide 11 / dodecabromide 12¹⁹, with the Schiff's base 13a-c.

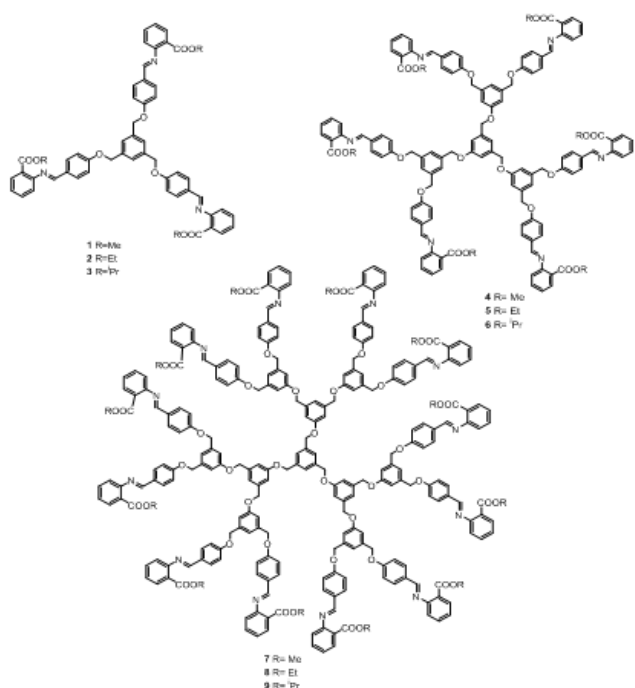
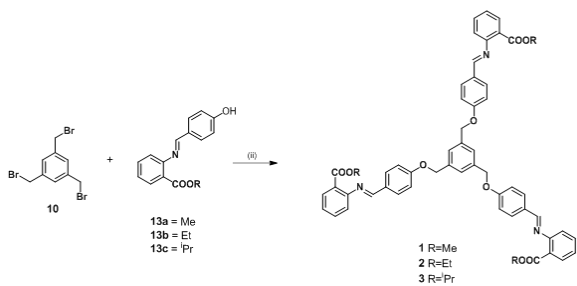
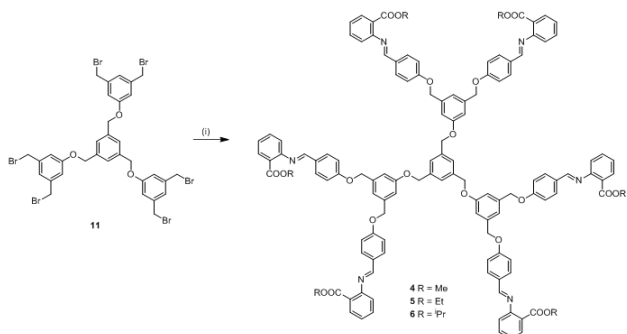


Figure 1. Frechet type dendrimers with Schiff's base at the periphery



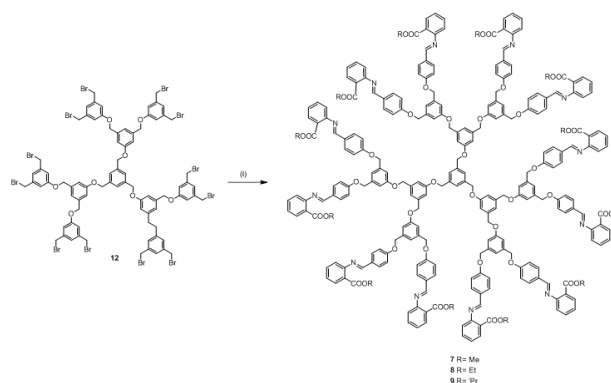
Scheme 1. Reagents and conditions: (i) K_2CO_3 , dry acetone, RT, 24 h, 1 (72 %), 2 (71 %), 3 (68 %)

In order to obtain the first generation dendrimers 4-6, six equivalents of 13a-c were treated with one equivalent of hexabromide 11 in dry acetone in the presence of K_2CO_3 give the dendrimers 4, 5 and 6 in 68 %, 66 % and 63 % yield respectively (Scheme 2).



Scheme 2 Reagents and conditions: (i) Methyl/ethyl/isopropyl (hydroxylbenzylidene amino) benzoate, K_2CO_3 , dry acetone, RT, 24 h, 4 (68 %), 5 (66 %), 6 (63 %)

In the 1H NMR spectrum of the dendrimer 4 the ester methyl protons appeared at δ 3.85 and the two distinct methylene protons at δ 5.10, 5.12 respectively in addition to other aromatic protons and the N=CH- proton appeared at δ 8.06.



Scheme 3 Reagents and conditions: (i) Methyl/ethyl/isopropyl (hydroxybenzylidene amino) benzoate, K_2CO_3 , RT, 24 h, 7 (55 %), 8 (54 %), 9 (54 %)

In ^{13}C NMR spectrum of dendrimer 4 the ester methyl carbon appeared at δ 51.5 and the two distinct methylene carbons at δ 69.8, 69.9 respectively. The N=CH- carbon appeared at δ 163.5 and the ester carbonyl at δ 168.6 in addition to other aromatic carbons. Similarly the structure of dendrimers 5 and 6 were confirmed from spectral and analytical data.

The second-generation dendrimers (G_2) 7-9 were obtained in 55 %, 54% and 54 % yields respectively by treating twelve equivalents of 13a-c with one equivalent of dodecabromide 12 in dry acetone in the presence of potassium carbonate (Scheme 3). The 1H NMR spectrum of dendrimer 7 showed the ester methyl protons at δ 3.90 and the two distinct methylene protons at δ 5.21, 5.23 respectively and the N=CH- proton at δ 8.08 in addition to other aromatic protons. The ^{13}C NMR spectrum of dendrimer 7 shows the ester methyl carbon at δ 51.2 and two distinct methylene carbons at δ 70.5, 70.7 respectively. The N=CH- carbon appeared at δ 161.7 in addition to other aromatic carbons. The structure of dendrimers 8 and 9 also was confirmed from the spectral and analytical data.

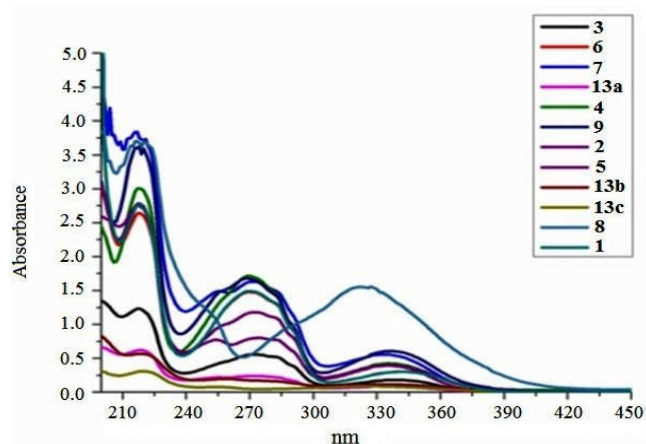


Figure 2. UV absorption of dendrimers 1-9 and the Schiff's bases 13a-c at 10^{-5} concentration in acetonitrile

The electronic absorbance spectra of dendrimers 1-9 were recorded in acetonitrile at a concentration of 10^{-5} M. In the absorption spectrum (Figure 2) three intense absorption bands appeared at 217, 268 and the other in between 342-344 nm. The absorption value of 217 nm is in good agreement with the reported value of methyl anthranilate (Rajakumar, 2014).

The intensity of absorption increases with increase in the number of anthranilates due to increase in the dendritic generations (Yohozowa *et al.*, 1998). Thus, the second generation dendrimers (G_2) 7-9 shows higher intensities than the zero and first-generation dendrimers. Fluorescence spectra of dendrimers 1-9 were recorded in acetonitrile at a concentration of acetonitrile at a concentration of $10^{-5}M$ by exciting at 220 nm. The emission spectrum of dendrimers 1-9 (Figure 3) shows various excitation values at 298, 342-345, 397 and 448 nm. The excitation maximum is at 448 nm with higher intensity. Irrespective of the different excitation values the emission occurs at 572 nm for all the synthesized compounds with differences in the intensities. Figure 3 clearly denotes that the intensity of fluorescence increases from zero-generation (G_0) to second generation (G_2) with the increase in the number of fluorophoric anthranilate, aromatic and imino units. The DPPH (1,1-diphenyl 2-picryl hydrazide) radical scavenging assay (Yohozowa *et al.*, 1998) is an easy, rapid and sensitive method to screen the antioxidant properties of the synthesized dendrimers.

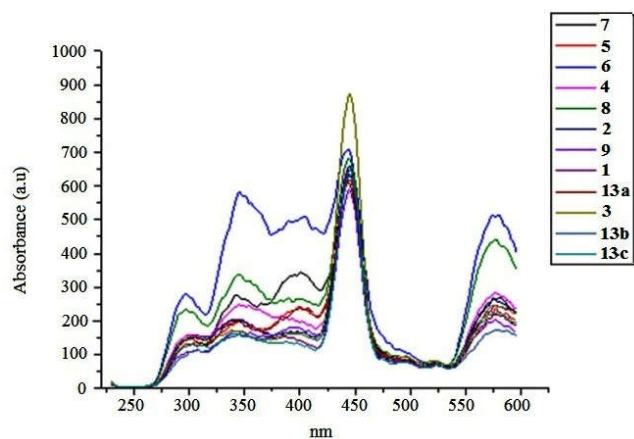


Figure 3. Emission spectra of dendrimers 1-9 and Schiff's bases 13a-c at $10^{-5} M$ concentration in acetonitrile at 220 nm

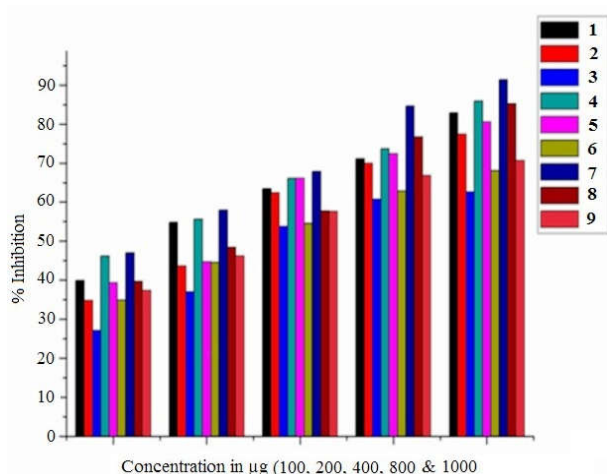


Figure 4, In-vitro free radical scavenging activity of dendrimers 1-9

DPPH scavenging assay reveals that the first and second generation dendrimer 4 and 7 respectively shows better antioxidant property.

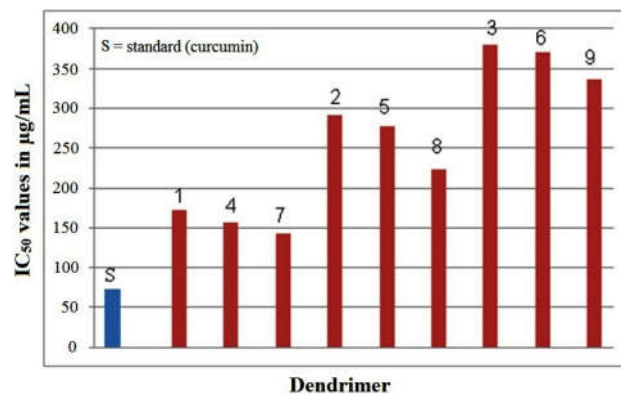


Figure 5. IC_{50} value of dendrimers 1-9 and curcumin

Figure 4 clearly indicates that the free radical scavenging property as measured by the DPPH method showed that percentage of inhibition increases with increasing concentration of the dendrimers which reveals its dose dependant antioxidant property. In comparison with the results of standard curcumin (Asouri *et al.*, 2013) (DPPH for curcumin is 90.36 %), the antioxidant activities of the first and second generation dendrimers 4 and 7 exhibited significant radical scavenging property. The antioxidant activity of the dendrimers increases with increase in the generation of dendrimers. The second generation dendrimer 7 shows better free radical scavenging activity at a concentration of $400 \mu g mL^{-1}$, with IC_{50} value of $143 \mu g mL^{-1}$ (Figure 5) which may be due to the presence of more number of anthranilate surface units which is usually called as valence effect in dendrimer chemistry.

Conclusion

In Conclusion, we have synthesized dendrimers with various Schiff's base derived from anthranilates as surface units by divergent approach via O-alkylation method. The absorption and emission studies reveal that absorption and emission values increases with increase in the generation of dendrimer. The anti-oxidant activity increases with increase in the generation of dendrimer. Among the dendrimers the second generation dendrimer 7 shows excellent scavenging property with an IC_{50} value of $143 \mu g mL^{-1}$.

EXPERIMENTAL SECTION

General Considerations

All melting points are uncorrected. The 1H NMR and ^{13}C NMR spectra were recorded on Bruker 300-MHz instrument in $CDCl_3$ solvent with tetramethylsilane (TMS) as a standard. Elemental analyses were carried out on a Perkin-Elmer CHNS 2400 instrument. Column chromatography was performed on silica gel (ACME, 100–200 mesh). Routine monitoring of the reaction was made using thin-layer chromatography (TLC) developed on 0.25 mm glass plates coated with silica gel-G (ACME) and visualized with iodine. UV-Visible spectra were recorded on Perkin Elmer Lambda 35 UV-Visible Spectrometer and the fluorescence spectra were recorded on Perkin-Elmer LF45 Luminescence spectroscopy.

General procedure for O-alkylation

To a solution of the hydroxy compound (2.1 mmol/4.2 mmol/8.4 mmol) in dry acetone was added tribromide/hexabromide/dodecabromide (0.7 mmol), K_2CO_3 (10.5 to 30.2

mmol) at room temperature for 24 h. The completion of the reaction was monitored by TLC and filtered. The solvent was then evaporated to dryness to afford the dendrimer as crude which was then purified by column chromatography on silica Gel (100-200 mesh) using hexane:CHCl₃ (3:2) as eluent.

Dendrimer 1

Brick Red solid. mp. 74-77 °C; Yield: 72 % ; ¹H NMR (300 MHz, CDCl₃): δ_H 3.84 (s, 9H), 5.17 (s, 6H), 6.60-6.65 (m, 6H), 7.04-7.07 (m, 6H), 7.21-7.26 (m, 3H), 7.49 (s, 3H), 7.81-7.85 (m, 9H), 8.27 (s, 3H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 51.5, 69.7, 115.1, 116.2, 116.7, 126.2, 130.3, 131.2, 131.7, 132.1, 134.1, 137.3, 150.5, 163.4, 168.6; m/z: 880 [M+1]

Dendrimer 2

Brick Red solid. mp. 76-80 °C; Yield: 71 % ; ¹H NMR (300 MHz, CDCl₃): δ_H: 1.37-1.43 (m, 9H), 4.37-4.43 (m, 6H), 5.15 (s, 6H), 7.17 (s, 6H), 7.26 (s, 3H), 7.29 (s, 6H), 7.54 (s, 3H), 7.83 (s, 9H), 8.27 (s, 3H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 14.3, 60.3, 61.6, 110.7, 113.6, 119.9, 123.3, 132.2, 138.3, 150.2, 151.8, 158.6, 164.7; m/z: 922 [M+1]

Dendrimer 3

Dark Red solid. mp. 84-87 °C; Yield: 68 % ; ¹H NMR (300 MHz, CDCl₃): δ_H 1.26-1.34 (m, 18H), 4.42 (br, 3H), 5.17 (s, 6H), 6.80 (br, 6H), 6.87 (br, 6H), 7.03 (br, 9H), 7.26-7.34 (m, 6H), 7.92 (s, 3H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 20.9, 61.2, 69.7, 107.0, 108.5, 126.6, 127.0, 127.3, 128.8, 132.5, 137.5, 159.3, 166.2

Dendrimer 4

Brick Red solid. mp. 112-116 °C Yield: 68% ; ¹H NMR (300 MHz, CDCl₃): δ_H 3.85 (s, 18H), 5.12 (s, 12H), 5.73 (s, 6H), 6.61-6.66 (m, 12H), 7.03 (s, 12H), 7.05 (s, 6H), 7.08 (s, 3H), 7.22-7.28 (m, 6H), 7.47 (s, 3H), 7.80-7.86 (m, 18H), 8.06 (s, 6H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 51.5, 69.8, 69.9, 110.8, 113.5, 115.1, 116.3, 116.7, 126.2, 130.3, 131.2, 132.0, 134.1, 138.3, 150.5, 159.8, 163.5, 168.6

Dendrimer 5

Brick Red solid. mp. 116-118 °C Yield: 64 % ; ¹H NMR (300 MHz, CDCl₃): δ_H 1.35-1.39 (m, 18H), 4.28-4.35 (m, 12H), 5.09 (s, 6H), 5.12 (s, 12H), 6.61-6.66 (m, 12H), 6.94-6.97 (m, 6H), 7.03-7.05 (m, 9H), 7.09 (s, 6H), 7.22-7.73 (m, 6H), 7.76-7.79 (m, 3H), 7.80-7.88 (m, 18H), 8.09 (s, 6H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 14.4, 60.4, 69.6, 69.8, 113.5, 115.2, 116.1, 116.3, 116.8, 130.2, 131.3, 132.1, 132.5, 134.1, 138.3, 150.4, 162.7, 164.6

Dendrimer 7

Blackish Red Gum; Yield: 55 % ; ¹H NMR (300 MHz, CDCl₃): δ_H 3.90 (s, 36H), 5.21 (s, 24H), 5.23 (s, 18H), 6.97 (s, 12H), 6.99-7.02 (m, 32H), 7.36-7.37 (m, 28H), 7.39-7.42 (m, 20H), 7.58 (s, 18H), 7.76-7.79 (m, 16H), 8.08 (s, 12H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 51.8, 70.5, 70.7, 116.4, 117.5, 117.7, 118.9, 121.7, 126.4, 131.3, 133.0, 133.9, 137.0, 148.5, 161.7, 163.2

Dendrimer 8

Blackish Red Gum; Yield: 54 % ; ¹H NMR (300 MHz, CDCl₃): δ_H 1.37-1.41 (m, 36H), 4.35 (br, 24H), 5.00 (s, 24H), 5.06 (s,

18H), 6.79 (s, 12H), 7.29 (s, 44H), 7.39 (s, 42H), 7.49 (s, 28H), 8.09 (s, 12H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 14.1, 60.1, 69.6, 69.7, 115.1, 116.2, 116.7, 126.0, 126.2, 130.3, 131.2, 131.7, 132.1, 134.1, 137.3, 150.5, 163.4, 168.6

Dendrimer 9

Blackish Red Gum; Yield: 50 % ; ¹H NMR (300 MHz, CDCl₃): δ_H 1.36-1.38 (m, 72H), 4.30-4.37 (m, 12H), 5.00 (s, 24H), 5.15 (s, 18H), 6.59-6.67 (m, 24H), 6.88 (br, 24H), 6.95-6.97 (m, 18H), 7.04 (br, 12H), 7.75-7.78 (br, 24H), 7.86-7.89 (m, 24H), 8.34 (br, 12H) ; ¹³C NMR (75 MHz, CDCl₃): δ_C 21.7, 61.2, 69.7, 70.1, 107.0, 108.0, 125.7, 126.3, 126.4, 126.6, 127.0, 128.8, 128.9, 132.5, 137.0, 137.1, 137.5, 159.3, 159.6, 159.7, 166.2

Absorption and Emission Studies

The electronic absorption spectra of the synthesized dendrimers were recorded on Perkin Elmer Lambda 35 UV-Visible Spectrometer at 10⁻³ M concentration in acetonitrile. Similarly the emission spectra were recorded on Perkin Elmer LF 45 Spectrometer at an excitation wavelength of 220 nm at 10⁻⁵ M concentration in acetonitrile.

Anti-oxidant Studies

The radical scavenging activities of the synthesized dendrimers were carried out by DPPH method. DPPH is a stable free radical with purple colour (absorbs at 517 nm). If the free radical gets scavenged, the colour of DPPH will change from purple to yellow and subsequently colourless depending on the strength of free radical scavenging activity. The reaction mixture containing 1.9 ml of DPPH solution (200 μM in ethanol) with different concentrations of the substance was shaken and incubated in dark for 30 min at room temperature. The resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using the formula

$$\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}$$

$$\text{Percentage Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

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Supplementary data

The supplementary data of this article can be accessed in the publisher's website.

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