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ORIGINAL ARTICLE

Antiradical Properties of *trans*-2-(4-substituted-styryl)-thiophene

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Abstract

2-substituted thiophene compounds with electron donating and electron withdrawing *p*-phenyl substitution were synthesized and studied their radical scavenging properties using DPPH assay and DFT method. It is shown that *p*-hydroxy and *p*-amino phenyl substituted compound exhibit radical scavenging activity. From DFT and radical scavenging studies, a correlation between IC_{50} with the bond dissociation enthalpy, proton affinity, ground state dipole moment and optical band gap of compound is found. Compounds **1**–**3** with electron withdrawing substituent (NO_2, CN, Cl) do not show any radical scavenging properties, whereas compounds **6**–**7** with electron donating substituent (OH, NH_2) show antiradical properties. Further, the antiradical activity is reduced drastically by replacing the -OH and -NH_2 with methoxy and -N-alkylating group respectively in **6** and **7.** The compound with *p*-hydroxy phenyl substitution, exhibits stronger antiradical activity as compared to the *p*-amino phenyl substitution due to smaller O-H bond dissociation energy as compared to the N-H bond. From DPPH and DFT studies, it is suggested that the radical scavenging activity in 2-substituted thiophene is occurred through proton transfer mechanism. The other possible SET, SPLET mechanisms are also corroborated.

Keywords Antioxidant ability · Thiophene · Phenol · Stilbene · Absorption · Fluorescence · Density functional theory · Bond dissociation energy · Ionization energy · Proton affinity

Introduction

Radical scavengers are active molecules, which play important role in many area of chemistry, biology and material science such as in food storage, cosmetic, pharmaceuticals, oil, rubber, petroleum products and in electronic device applications [1–4]. Some of the well known naturally occurring radical scavengers are flavonoids [5–11], glutathione [12, 13], vitamin A, vitamin C [14, 15], vitamin E [16], uric acid [17], caffeic and ferulic acids [18, 19], β-carotene [10], curcumin [20–22], bilirubin [23]. However, some of these anti-radicals like β-carotene, vitamin A, and vitamin E have no suppressing effect towards abnormal biological activity· [24–26]. Thus, in recent years, there have been growing interest in developing novel radical scavengers that fulfill the need for industrial and pharmaceutical applications. Many synthetic antioxidants were designed and synthesized to improve the radical scavenging properties that can be used in biological and industrial applications [27–34]. For example, antiradicals based on stilbenoid [35–38], butylated hydroxy phenyl compounds, BHA, BHT [1], dihydroquinoline ethoxyquin [39] are useful for above applications. Recently, some of the thiophene based hybrid compounds, such as dithinyldene cyclohexanone (IC_{50}: 4 μM) [40], thiophene based schiff base (IC_{50}: 5 μM) [41] are known to exhibit remarkably higher radical scavenging properties and useful in wide range of applications [42–46]. Thus, in order to grasp the basic fundamental of radical scavenging activity, studies on the antiradical properties on various donor and acceptor substituted ethenyl thiophenes (**1**–**8**) (Fig. 1) were carried out using DPPH assay and DFT methods.

Anamika Gusain and Naresh Kumar contributed equally to this work.

Highlights
Thiophene compounds with *p*-hydroxy and *p*-amino phenyl substitutent, exhibit antiradical activity with IC_{50} range from 45 μM to 165μM . The activity is comparable to vitamin E (IC_{50} : 26 μM)
Correlation between the anti-radical activity with the ground state dipole moment, bond dissociation enthalpy, ionization potential and proton affinity of thiophene compound is elucidated.
In thiophene compounds, the radical scavenging activity is predominantly occurred through hydrogen atom transfer mechanism. The other possible mechanisms such as SET, SPLET are also discussed.

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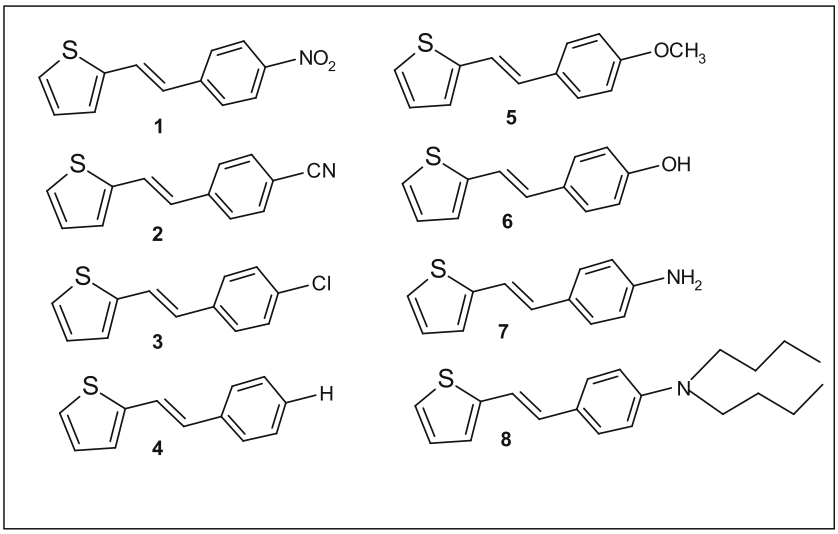


Fig. 1 Structure of compounds **1**–**8**

It is shown that the thiophene compounds with donor substituent (-OH and -NH_2) exhibit antiradical properties, whereas the activity is reduced in presence of withdrawing substituent. In the presence of thiophene compound, the DPPH radical is mostly quenched through proton transfer mechanism. The results are also supported by various thermodynamic parameters obtained through TDDFT calculation.

Experiment

Material and Methods

Chemicals are purchased from M/s. Sisco Research Laboratory. Radleys make, Carousel 6 plus reaction station was used for the synthesis of compounds **1–8**. Perkin Elmer Lambda 750 UV/VIS/NIR spectrophotometer is used to record the absorption spectra and Perkin Elmer LS-55 fluorescence spectrophotometer is used to record the fluorescence spectra using a red PMT detector system. FTIR spectra were recorded on a Impact Nicolet-400 spectrophotometer using KBr discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL 500 MHz FTNMR instruments. GC-MS spectra were recorded on a GCD 1800A Hewlett packard GC-mass spectrometer. CHNS analyses were carried out on a Theoquest CE instrument 1112 series CHNS auto analyzer. Melting points were determined on a Lab India make melting point apparatus. For spectroscopic studies, UV grade solvents were used.

Synthesis of Compounds 1–8

The synthetic scheme of all compounds are shown in Fig. 2. The substituted *p*-phenyl ethenyl-*E*-thiophenes (**1, 3–6**) were synthesized by the condensation of *p*-substituted phenyl acetic acid with the corresponding 2-formylthiophene (2:1 M ratio) in presence of pyridine-piperidine mixture as described earlier [47–54], e.g. typical synthetic protocol for compound **1** is as follows: 2-formyl thiophene (0.93 mL, 0.01 mol) was refluxed with mixture of 10 mL of freshly distilled pyridine, 0.6 mL of piperidine and 3.62 g, (0.02 mol) of *p*-nitrophenyl acetic acid at 100 °C for eight hours and the progress of the reaction was monitored by thin layer chromatography. The reaction mixture was then cooled and poured in ice-cold water and treated with 100 mL of diluted hydrochloric acid to remove excess of pyridine from the reaction mixture. A yellow colored product was extracted in chloroform and purified by column chromatography using 2% ethyl acetate in petroleum ether as the eluting solvent, when the desired compound was obtained in 30% yield. Compound **7** was obtained through reduction reaction of **1** [52]. For this purpose, ethenyl thiophene **1** in ethanol was refluxed in presence of aqueous ferrous sulfate and ammonia solution at 100 °C for 3 h. All the products were purified by column chromatography using 2–10% ethyl acetate in petroleum ether (60–80 °C) as the eluting solvent. Compound **8** was synthesized through alkylation of compound **7** in presence of potassium-*tert*-butoxide. Compound **2** was prepared through condensation of 2-formyl thiophene and corresponding phosphite using Wadsworth-Emmons reaction [52, 55, 56]. All compounds show satisfactory physicochemical data (UV-Vis, FTIR, ^{1}H and ^{13}C NMR, GC-MS and CHNS analysis).

Radical Scavenging Activities

For radical scavenging activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried out by following the protocol described elsewhere [57]. In a typical experimental procedure, 100 μM concentration of DPPH solution was prepared by adding 0.4 mL of 1×10^{-3} M methanolic solution of DPPH. A varying concentration of testing compound (0 to

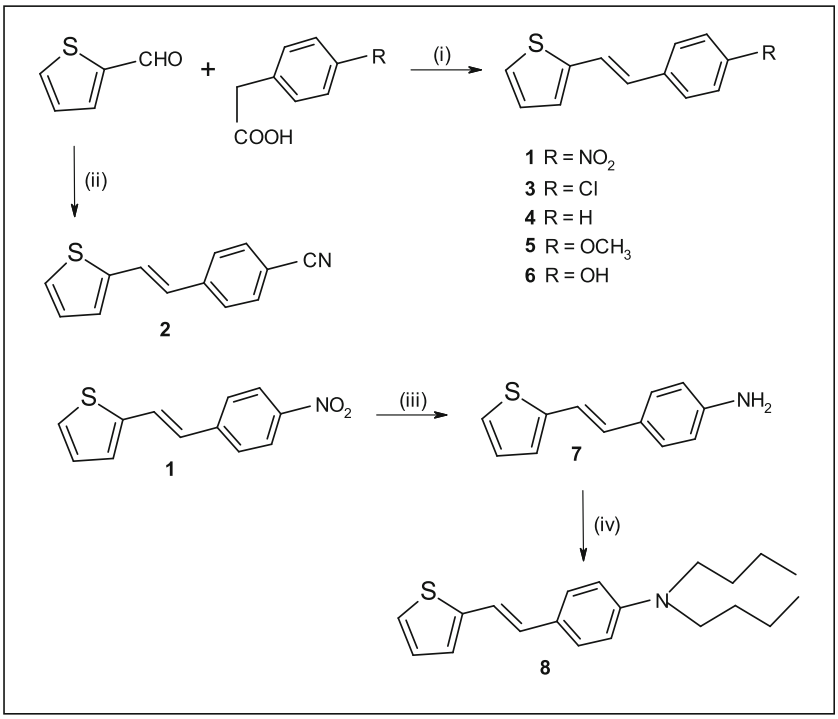


Fig. 2 Synthetic scheme for ethenyl thiophenes (**1**–**8**); (i) pyridine-piperidine, 100 °C, 8 h; (ii) (a) *p*-cyano benzyl bromide, $P(OEt)_3$, 150 °C, 3 h; (b) DMF, NaH, 0 °C, 1 h; (iii) FeSO4, aqu. NH_3, ethanol, 100 °C, 2 h; (iv) Potassium-*tert*-butoxide, *tert*-butyl alcohol, Butyl bromide, r.t. 3 h

400 μM) was then added to DPPH solution depending upon the antiradical activity. The decrease in the absorbance of DPPH radical at 517 nm was then measured at regular interval of time (0–45 min duration). The DPPH solution with standard antioxidant, vitamin E is used as a positive control. All the experiments were performed in triplicate and the average of absorbance was taken for calculating the inhibition concentration. The 50% inhibition concentration (IC_{50}) is the concentration of antioxidant at which the 50% of absorbance of DPPH radical is quenched with respect to the control (A_{blank}). The IC_{50} is calculated from the plot of % inhibition vs. concentration of antioxidant, and using the eq. 1.

$$\%\text{Inhibition of DPPH free radical} = \left[\left\{A_{blank} - A_{sample}\right)/(A_{blank})\right] \text{ x } 100 \quad (1)$$

where A_{blank} = Absorbance of DPPH radical in absence of antioxidant; A_{sample} = Absorbance of DPPH radical in presence of varying concentration of antioxidant.

Table 1 Ground state dipole moment (μ_g), absorption (λ_{abs}) and fluorescence wavelength maximum (λ_{em}), extinction coefficient (ε), optical band gap, DPPH radical inhibition concentration (IC_{50}) of ethenyls thiophenes in methanol

Com	μ_g (Debye)	λ_{abs} (nm)[a]	λ_{em} (nm)[a]	ε (M^{-1} cm^{-1})	Band Gap(eV)[b]	Band Gap(eV)[c]	Band Gap(eV)[d]	IC_{50} (μM)
1 -NO_2	6.65	372	614	21,500	2.89	3.14	3.51	–
2 -CN	5.60	340	412	39,000	3.28	3.31	3.73	–
3 -Cl	1.87	327	384	30,400	3.46	3.49	3.90	–
4 -H	0.34	321	385	21,000	3.51	3.55	3.98	–
5 -OCH_3	1.19	332	387	44,500	3.43	3.44	3.85	–
6 -OH	1.42	325	378	24,280	3.47	3.53	3.90	45
7-NH_2	3.00	343	435	27,500	3.20	3.19	3.75	165
8-NR_2	3.48	367	420	12,400	2.88	3.16	3.64	322

[a] Experimentally obtained; Vitamin E (IC_{50}, 26 μM, μ_g: 0.80); ascorbic acid (IC_{50} ~ 11 μM); 4-hydroxy stilbene (IC_{50} ~ 24 μM);

[b] Obtained through Tauc plot,

[c] Obtained through intersection of absorption and fluorescence spectra,

[d] Obtained through DFT

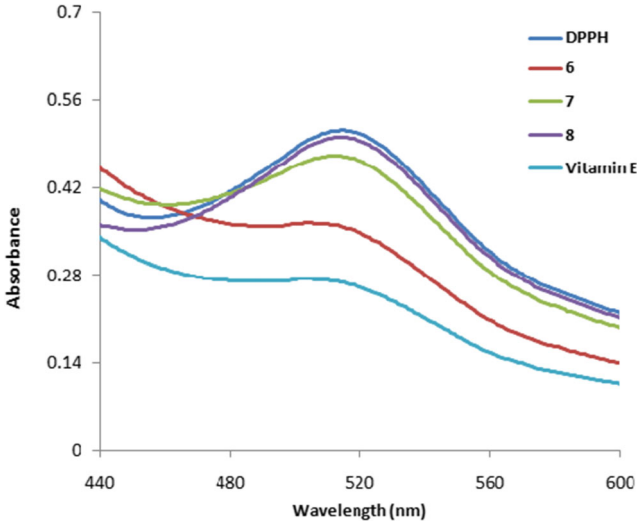


Fig. 3 A typical absorption spectra of 100 μM of DPPH radical alone and in presence of 25 μM concentration *of* ethenyl thiophenes **6–8** and vitamin E

Time Dependent Density Functional Theory (TDDFT)

For the calculation of thermodynamic parameters (BDE, IP, PA, PDE, ETE), the ab initio quantum chemical software package ORCA is used [58]. The ground state dipole moment, absorption and fluorescence wavelength maximum, the vertical excitation energy and oscillator strength (f) is computed using time-dependent density functional theory (TDDFT) [59, 60]. The ground and excited state of the neutral, free radical, cationic radical and anionic thiophenes are optimized through B3LYP and BLYP functional using def2-SVP and aug-cc-pVDZ basis set respectively [61]. The minimized geometry is further confirmed by vibrational analysis, resulting in no imaginary frequencies. This geometry is used as the input for further calculations to obtain the frontier molecular orbitals (FMOs), UV-Vis and fluorescence spectra. The TDDFT

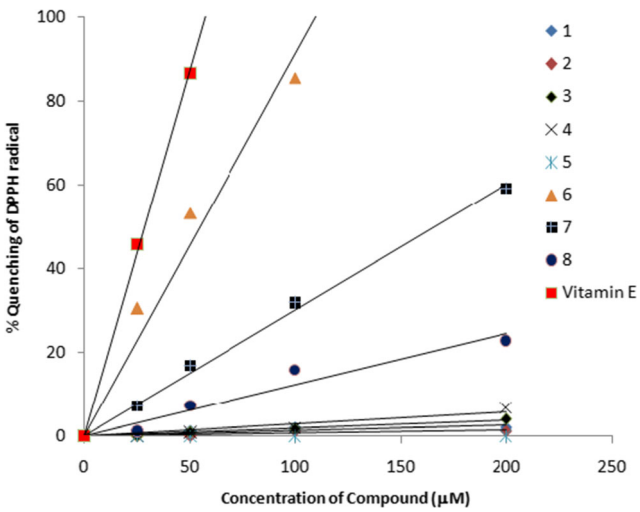


Fig. 4 Plot of % of DPPH radical quenching vs. concentration of ethenyl thiophenes **1–8**

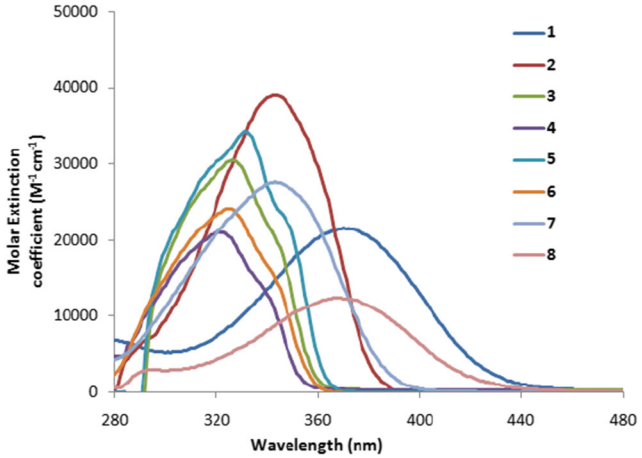


Fig. 5 Absorption spectra of compounds **1–8** in methanol

predicted bond dissociation energy is most reliable and comparable with other computational methods [62, 63]. The DFT method is validated using known compounds, vitamin E, 4-hydroxy stilbene, 2,4,6-tri-*tert*-butylphenol, hydrogen radical, whose results are well matched with the previously reported experimental and theoretical data [64–66].

Results and Discussion

Radical Scavenging Properties of Ethenyl Thiophene

The radical scavenging properties of thiophene compounds (**1–8)** are carried out using DPPH assay in methanol. In general, the DPPH free radical is quenched at 517 nm in presence of an antioxidant. It is observed that the absorbance of DPPH radical is quenched in the presence of thiophene compounds **6–8**, whereas, the absorbance of DPPH radical is almost

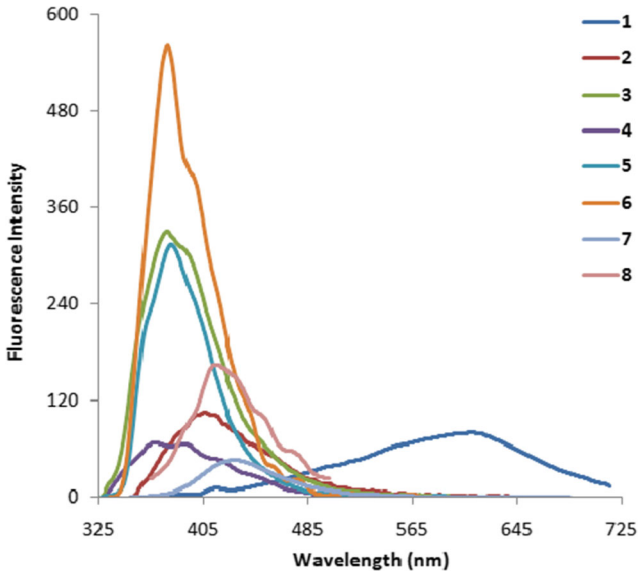


Fig. 6 Fluorescence spectra of compounds **1–8** in methanol

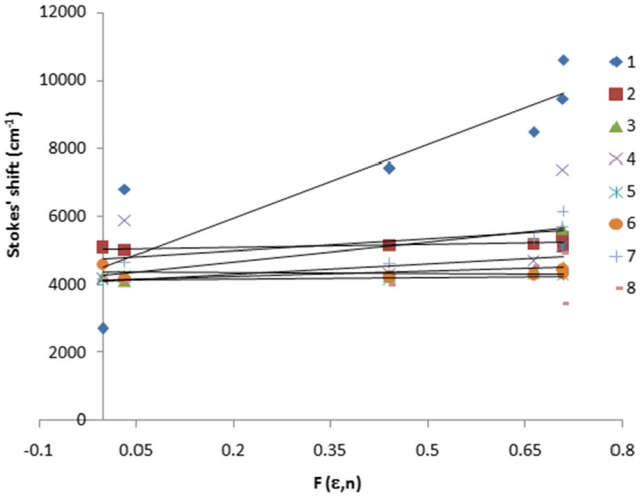


Fig. 7 McRay Plot, Stokes' shift vs. solvent polarity parameter, F(ε,n) of **1**–**8**

unaffected in presence of other thiophene compounds **1**–**5**. The % of quenching of DPPH radical is obtained from the plot of % of inhibition vs. compound concentration (Fig. 3 and 4**)**. The hydroxy (**6**) and amino (**7**) substituted thiophene compounds exhibit radical scavenging properties with IC_{50}: 45 μM and 165 μM respectively (Table 1). Upon O-alkylation and N-alkylation of hydroxy and amino functional group respectively, the radical quenching activity of **5** and **8** is drastically reduced (IC_{50} ~ 322 μM for **8** and no activity for **5**). On the other hand, thiophene compounds (**1–5)** do not show any radical scavenging activity. Thus, the hydroxy and amino compounds are capable of transferring hydrogen to the DPPH radical and subsequently, the quenching of DPPH radical is occurring. These indicate that the thiophene compounds quench the DPPH radical predominantly through the hydrogen atom transfer (HAT) mechanism, which leads to the formation of neutral DPPHH molecule.

Correlation of Antiradical Activity with Optical Properties

In order to understand the anti-radical activity in detail, the optical properties of the molecule are studied. The absorption and fluorescence spectra of **1**–**8** were recorded in solvent of varying polarity (**Fig. S1-S2**). It is shown that the absorption (λ_{abs}) and fluorescence wavelength (λ_{em}) of all these thiophenes are red shifted from non-polar solvent *n*-hexane to polar solvent DMF (**Table S1**). The molar extinction coefficient (ε) of thiophene compounds lies in between 10,000 M^{-1} cm^{-1} to 30,000 M^{-1} cm^{-1}. These indicate the $\pi \rightarrow \pi^*$ nature of transition in thiophene compounds **1**–**8** (Table 1, Figs. 5 and 6). The λ_{abs} is moderately red shifted from non-polar solvent, *n*-hexane to polar solvent, DMF, by 15 nm, 3 nm, 4 nm, 4 nm, 5 nm, 3 nm, 17 nm and 17 nm, whereas the λ_{em} is red shifted by 156 nm, 6 nm, 8 nm, 13 nm, 9 nm, 0 nm, 45 nm and 24 nm for **1**–**8** respectively. As compared to 2-[phenyl ethenyl-*E* thiophene](**4**), the λ_{abs} and λ_{em} of 2-[4-nitro phenyl ethenyl-*E*-thiophene](**1**) are red shifted by 51 nm and 229 nm respectively. Similarly, λ_{abs} and λ_{em} of **8** are red shifted by 45 nm and 35 nm, whereas a minimal change of 0–4 nm is observed for thiophenes **3**–**6**. The λ_{em} is highly sensitive to solvent polarity and *p*-phenyl substituent. The large red shift of λ_{em} in **1** suggest the involvement of charge transfer excited state for **1**, whereas, a moderate red shift in **7** and **8** suggest a partial charge transfer in the excited state of amine compounds **7** and **8**. These type of charge transfer phenomena are very common in nitro and amine compounds [51–54]. The change in excited state dipole moment is obtained for **1**–**8** as 13.85 Debye, 2.54 Debye, 2.47 Debye, 4.41 Debye, 1.91 Debye, 1.78 Debye, 6.52 Debye and 5.20 Debye respectively using McRay Plot (Fig. 7, **Table S2**). These further indicate that compound **1** is highly dipolar and exhibits charger transfer excited state, whereas amine

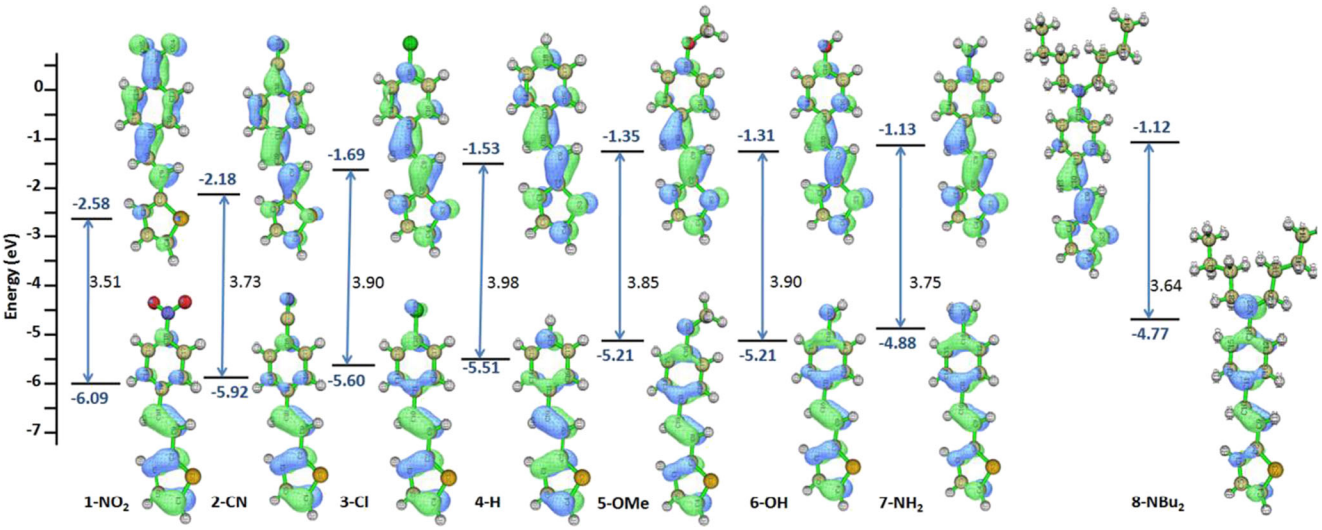


Fig. 8 TDDFT computed HOMO-LUMO energy of ethenyls **1**–**8**

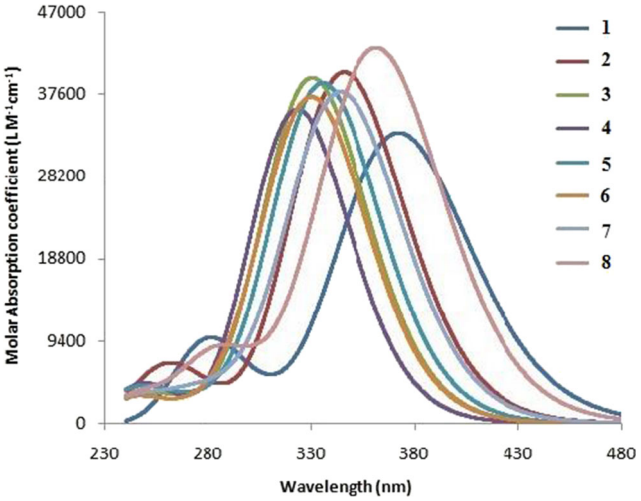


Fig. 9 TDDFT computed UV-Vis absorption spectra of compounds **1**–**8**

compounds **7** and **8** undergo partial charge transfer and other compounds have non-polar excited state.

The optical band gap of these compounds were obtained using both experimental and theoretical methods (Table 1, **Table S3, Fig. S3**). In all the methods, the optical band gap follows the similar trend. The ground and excited state of thiophene compound are stabilized more in presence an electron withdrawing *p*-phenyl substituent. However, as compared to the unsubstituted thiophene compound **4**, the optical band gap of substituted compound is decreased either by an electron withdrawing or electron donating *p*-phenyl substituent **(**Table 1**)**. The optical band gap obtained through DFT method is little larger than the experimental method. The effect of solvent is not taken in to account in DFT method and thus, there is a less stabilization of ground and excited state in DFT method. This leads to a shorter emission wavelength with little larger optical band gap **(**Figs. 8, 9, 10 and 11, **Fig. S3**).

The HOMO and LUMO energy of ethenyl thiophene are gradually increased in presence of an electron donating substituent. For nitro, cyano and chloro compounds (**1**–**3**)**,** a

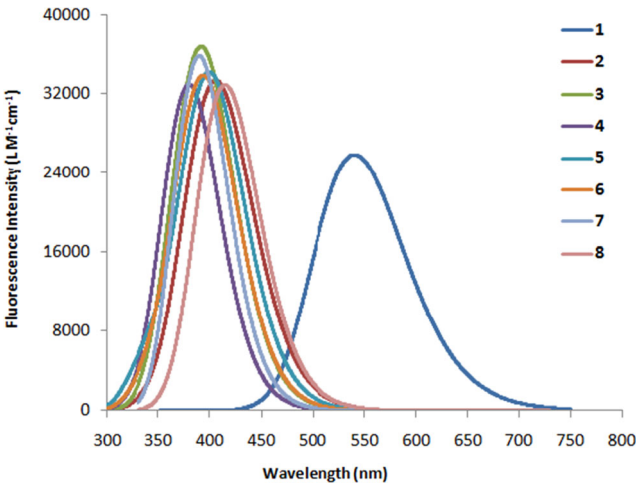


Fig. 10 TDDFT computed fluorescence spectra of compounds **1**–**8**

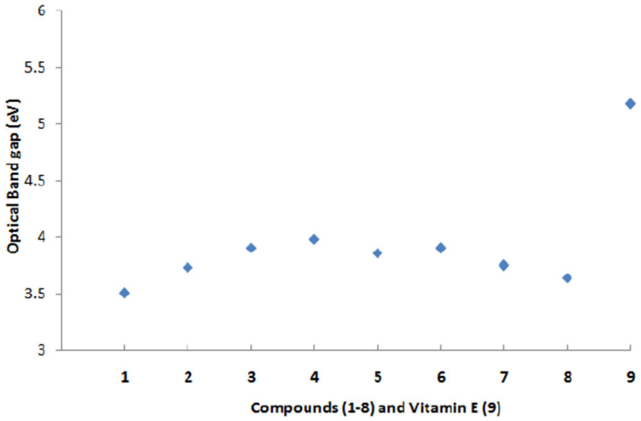


Fig. 11 Optical band gap of **1**–**8** and vitamin E (**9**)

lowest HOMO energy is observed (**1**: HOMO: −6.09 eV, LUMO: −2.58 eV; **2**: −5.92 eV, LUMO -2.18 eV; **3**: −5.60 eV, LUMO -1.69 eV; **4**: −5.51 eV, LUMO -1.53 eV), whereas a highest HOMO energy is computed for hydroxy and amine compounds (**6:** HOMO: −5.21 eV, LUMO -1.31 eV, **7**:HOMO: −4.88 eV, LUMO -1.13 eV, **8:** HOMO: −4.77 eV, LUMO: −1.12 eV).

Similarly, the ground state dipole moment is computed for **1**–**8** using DFT method. It is shown that **1, 2**, **7** and **8** exhibit large ground state dipole moment (**1**: 6.65 Debye, **2**: 5.60 Debye, **7**: 3.00 Debye, **8**: 3.48 Debye), whereas other thiophene compounds (**3**–**6**) show a small dipole moment (Vitamin E: 0.80 Debye, **3**: 1.87 Debye, **4**: 0.34 Debye, **5**: 1.19 Debye, **6**: 1.42 Debye). As compared to **4**, the dipole moment is increased with increasing the electron withdrawing capacity of *p*-pheny substituent (**1**-NO_2: 6.65 Debye, **2**-CN: 5.60 Debye, **3**-Cl: 1.87 Debye, **4**-H: 0.34 Debye) and also with increasing the electron donating capacity of *p*-phenyl substituent (**8**-NR_2: 3.48 Debye, **7**-NH_2: 3.00 Debye, **6**-OH: 1.42 Debye, **4**-H: 0.34 Debye) (Fig. 12).

Thus, thiophene is acting as an electron donor or electron acceptor depending upon the nature of *p*-phenyl substitution. Interestingly, thiophenes with electron withdrawing substituent (NO_2, CN, Cl) do not show any radical scavenging

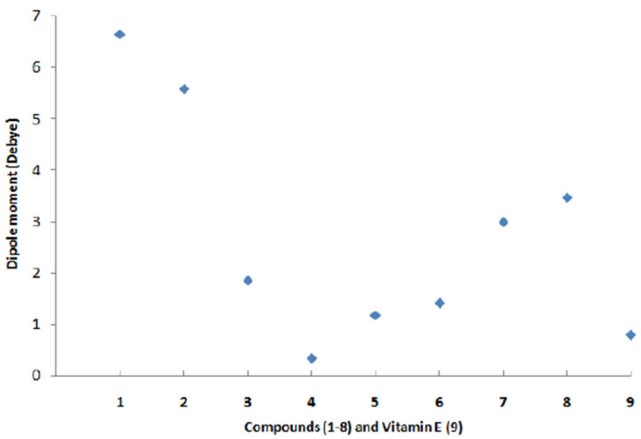


Fig. 12 Ground state dipole moment of **1**–**8** and vitamin E (**9**)

Table 2 TDDFT computed hydrogen bond dissociation (BDE), ionization potential (IP), proton dissociation (PDE), proton affinity (PA) and electron transfer (ETE) energy (Kcal/mol) of ethenyl thiophenes (**1–8**)

Compound	BDE	ΔBDE	IP	ΔIP	PDE	ΔPDE	PA	ΔPA	ETE	ΔETE
Vitamin E	82.9	0	157.4	0	236.3	0	365.3	0	29.1	0
1-NO_2	–	–	177.0	19.6	–	–	–	–	–	–
2-CN	–	–	172.6	15.1	–	–	–	–	–	–
3-Cl	–	–	165.1	7.7	–	–	–	–	–	–
4-H	–	–	164.5	7.0	–	–	–	–	–	–
5-OCH_3	–	–	155.5	−1.9	–	–	–	–	–	–
6-OH	89.5	6.6	156.6	−0.7	243.7	7.4	355.6	−9.68	45.5	16.4
IC_{50}: 45 μM										
7-NH_2	99.1	16.1	148.3	−9.2	261.7	25.4	379.5	14.2	31.1	2.0
IC_{50}: 165 μM										
8-NR_2	–	–	141.5	−15.8	–		–		–	–
IC_{50}: 322 μM										

$\Delta BDE = BDE\text{-}BDE_{vitamin\ E}$; $\Delta IP = IP\text{-}IP_{vitamin\ E}$; $\Delta PA = PA\text{-}PA_{vitamin\ E}$; $\Delta PDE = PDE\text{-}PDE_{vitamin\ E}$; $\Delta ETE = ETE\text{-}ETE_{vitamin\ E}$

properties. On the other hand thiophene compounds (**6–8**) with electron donating substituent such as amine and hydroxy, exhibit anti-radical properties. The radical scavenging efficacy of such compounds, however, is decreased further upon alkylation. The order of radical scavenging activity is: Vitamin E > **6**-OH > **7**-NH_2 > **8**-NR_2. Thus, in these compounds, the radical scavenging activity is directly related to the optical band gap (Fig. 11), whereas inversely related to the dipole moment of the thiophene compounds (Fig. 12). The anti-radical mechanism is very complex and in order to understand the mechanism in detail, the thermodynamic parameters such as BDE, IP, PA, PDE, ETE of the molecules (**1–8**) are calculated using the eq. 2–6 and the data is shown in Table 2.

$$BDE = E_{Ar-X\bullet} + E_{H\bullet} - E_{Ar-XH} \quad (2)$$

$$IP = E_{Ar-X^{\bullet+}H} - E_{Ar-XH} \quad (3)$$

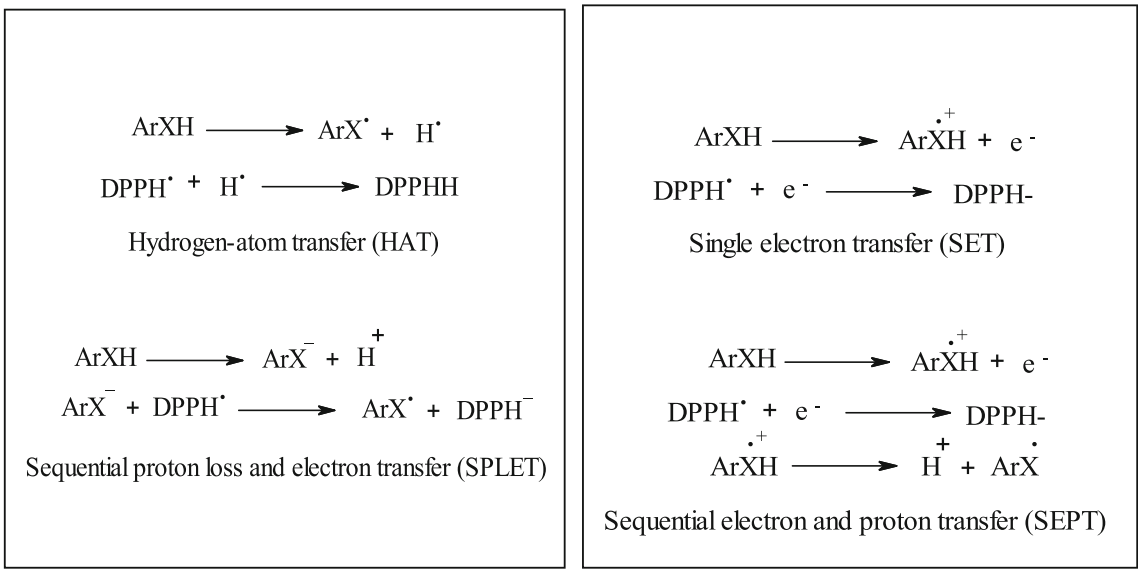
$$PA = E_{Ar-X^{-}} + E_{H^{+}} - E_{ArXH} \quad (4)$$

$$PDE = E_{Ar-X^{\bullet}} + E_{H^{+}} - E_{ArX^{\bullet+}H} \quad (5)$$

$$ETE = E_{Ar-X^{\bullet}} + E_{e^{-}} - E_{Ar-X^{-}} \quad (6)$$

where $E_{Ar\text{-}XH}$, $E_{Ar\text{-}X\bullet}$, $E_{Ar\text{-}X^{\bullet+}H}$, $E_{Ar\text{-}X^{-}}$, $E_{H\bullet}$, $E_{H^{+}}$, $E_{e^{-}}$ are the enthalpies of Ar-XH, Ar-$X^{\bullet}$ radical, Ar-$X^{\bullet+}H$ cationic radical, anion Ar-X^{-}, H radical, H^{+} cation, electron respectively [67–80].

Many radical scavenging mechanisms are well known in the literature [67–80]. These include hydrogen atom transfer (HAT) [67–70], single electron transfer (SET) [70–73], radical adduct formation (RAF) [74], sequential proton loss and electron transfer (SPLET) [75, 76], sequential electron proton transfer [SEPT] [77–79], sequential proton loss hydrogen

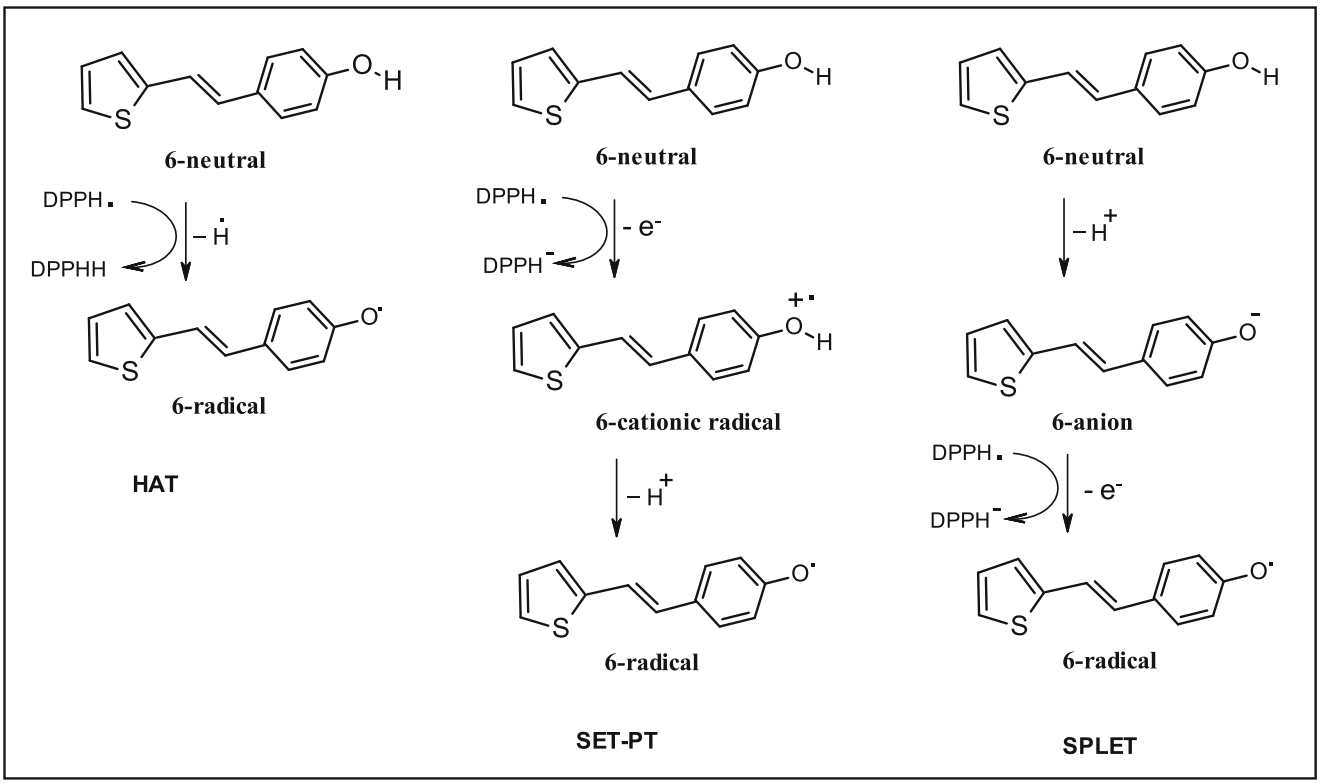


Scheme 1 Some of the plausible radical scavenging mechanisms, HAT, SET, SPLET, SEPT.

atom transfer (SPLHAT) [80] etc. (Scheme 1). The HAT mechanism is associated with the hydrogen bond dissociation enthalpy (BDE), whereas, SET is associated with ionization potential (IP) of the antiradical. Similarly, SPLET is associated with proton affinity (PA) and electron transfer enthalpy (ETE), whereas SPET is associated with both IP and PA of the antiradical. Thus, thermodynamic parameters, such as BDE, IP, PA, PDE, ETE provide most valuable information in predicting the plausible mechanism.

From Table 2, the IP energy of **1–4** is found to be very large (IP: 164.17–177.03 Kcal/mol), as compared to **5–8** (IP: 141.55–156.63 Kcal/mol). In **5–8** and vitamin E, the trend of IP energy is vitamin E ~ **6** > **7** > **8**. and the trend of radical scavenging efficacy is: vitamin E > **6** > **7** > **8.** It is known that electron withdrawing substituent stabilize the neutral molecule, and destabilize the radical and radical cation, which leads to a higher IP energy [69, 70]. On the other hand, electron donating substituent stabilized the radical, radical cation and destabilized the neutral molecule, which leads to decrease in the IP energy [70, 72]. Thus, compound with higher antiradical activity should have a lower IP energy to act through the SET mechanism [71, 72]. Similarly, the proton dissociation energy (PDE) provides useful information for the later step of SET-PT mechanisms [81] (Scheme 2). The PDE of cationic radical of vitamin E, **6** and **7** is found as 236 kcal/mol, 243 kcal/mol, and 261 kcal/mol respectively. Thus PDE is larger for thiophene with strong electron donating amine substituent and it requires higher energy for the dissociation of proton from the radical cation intermediate. Thus, it is suggested that these compounds may not follow anti-radical activity through SET or SET-PT mechanism.

To confirm the hydrogen atom transfer (HAT) mechanism, the BDE is calculated for **6**–**7** and vitamin E. The O-H and N-H bond dissociation energy ($BDE_{O\text{-}H}$, $BDE_{N\text{-}H}$) is little larger for **6** and **7** (89 Kcal/mol for **6** and 99 Kcal/mol for **7**) as compared to the vitamin E (82.97 Kcal/mol). In **6** and **7,** the O-H and N-H bond dissociation energy is increased by 6 Kcal/mol ($\Delta BDE_{O\text{-}H}$) and 16 Kcal/mol ($\Delta BDE_{N\text{-}H}$), as compared to vitamin E respectively. The trend of BDE is: **7** > **6** > vitamin E and the radical scavenging activity is also reduced in the order Vitamin E > **6** > **7**. This trend of BDE and anti-radical activities is in accord to the HAT mechanism. Further, replacing the OH and NH_2 with methoxy (**5**: $-OCH_3$) and di-alkyl (**8**: $-N(C_4H_9)_2$] group, results in the reduction of anti-radical activity. These suggest that thiophene compounds exhibit antiradical activities through hydrogen atom transfer (HAT) mechanism (Scheme 2). In general, the antiradical activity of phenol and amine compounds occur through H atom transfer mechanism (HAT). Such compounds have smaller BDE with more stabilized phenoxyl or imine radical [62, 67–69, 81–88]. In thiophene compounds, **6** and **7**, the BDE of N-H bond is 10 kcal/mol higher than O-H bond. This indicates that the imine radical is less stable than the phenoxyl radical



Scheme 2 Possible antiradical mechanisms in ethenyl thiophenes

and thus, compound **7** exhibits less anti-radical activity compared to the hydroxy compound **6**.

The other possible mechanism is the sequential proton loss and electron transfer (SPLET) mechanism via the anionic intermediate (Scheme 2**)**. In this mechanism the role of PA is important. The PA for phenyl N-H and phenol O-H bond is 379 kcal/mol and 355 kcal/mol respectively. In the present thiophene compounds, the trend of PA energy is: **7** > **6** and the antiradical activity is in the order **6** > **7**. If the anion generate through proton abstraction by following the SPLET mechanism as shown in Scheme 2, the phenoxyl anion of ethenyl thiophene could be stabilized more as compared to the aminyl anion and consequently decrease in PA for **6** as compared to **7** and increase in the antiradical activity of **6**. However, the ETE of phenoxyl anion (**6**) is 14 Kcal/mol is larger compared to amine anion (**7**), which is the second step of SPLET pathway (ETE: 45 kcal/mol for **6,** 31 kcal/mol for **7**). A more stabilized anion requires higher energy to transfer electron to the free radical [69, 86]. Thus, SPLET mechanism can be ruled out. Thus, in **6** and **7** as the BDE is smaller than IP and PA energy, the antiradical activity could be through thermodynamically controlled HAT mechanism.

Conclusion

In summary, the antiradical activities of *p*-phenyl substituted ethenyl thiophenes were studied using DPPH assay and density functional theory. It is shown that ethenyls with strong electron-donating substituent (NH_2, OH) exhibit antiradical activity, whereas ethenyls with electron withdrawing substituent do not show antiradical activity. From the studies on the optical properties, it is shown that ethenyl thiophene with small ground state dipole moment and large optical band gap, exhibits good antiradical properties.

From the studies on the thermodynamic parameters, it is shown that amine and hydroxy substituted thiophenes have smaller bond dissociation enthalpy (BDE) as compared to ionization energy (IE) and proton affinity (PA). Thus, for quenching of free radical, these compounds follow the thermodynamically controlled HAT mechanism. Ethenyls with electron withdrawing substituent (NO_2, CN, Cl) do not show any antiradical properties owing to higher IP energy and lack of loosely bound hydrogen atom. It is also noteworthy to mention that the antiradical mechanism is very complex and hence, kinetic and radical quenching studies can provide more detail insights into the mechanism of action. Overall, the present ethenyl thiophene exhibits substituent dependent antiradical activity, which is interesting. These result, however, provide a very useful information in designing future molecules that exhibit efficient antiradical activities.

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Author Contributions PKH, AG, NK synthesized and characterized the compounds using ^{1}H and ^{13}C NMR, GC-MS, FTIR techniques. PKH, AG, NK carried out the absorption, fluorescence measurement and analyzed the data. PKH, AG, NK and GP measured the antiradical activity. PKH and JK designed and JK carried out the DFT calculation. PKH, AG and NK wrote the paper.

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