

## Synthesis and biological evaluation of novel 1,5-benzothiazepin-4(5H)-ones as potent antiangiogenic and antioxidant agents

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### ABSTRACT

Novel 1,5-benzothiazepin-4(5H)-one derivatives (**8a-8g**) have been synthesized by intramolecular cyclization of **6** using propyl phosphonic anhydride (T3P) as a cyclodehydrating agent. The anti-angiogenic and anti-oxidant properties of the new derivatives were then evaluated. Compounds **8b**, **8d**, **8e**, **8f** and **8g** exhibited very good inhibition of capillary proliferation, thus proving their anti-angiogenic properties. In addition, the *in vitro* antioxidant activities of these compounds were evaluated using diphenyl picryl hydrazine (DPPH), OH<sup>•</sup>, metal ion-chelating and NO (nitric oxide) assays, and the results compared with butylated hydroxytoluene (BHT), a well known anti-oxidant. Compounds **8a-8g** showed excellent free-radical scavenging activities in the metal ion-chelating assay, and compounds **8f** and **8g** showed nitric oxide radical scavenging properties at a concentration of 20-100 µg/mL, and are thus more potent than BHT.

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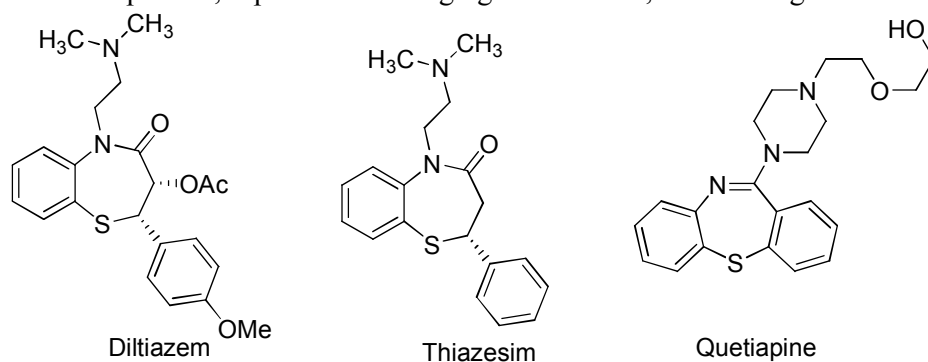
## 1. Introduction

Angiogenesis is the development of new blood vessels from pre-existing vasculatures and this has been well recognized as an essential hallmark for the growth, invasion and metastasis of tumors.<sup>1</sup> Deregulation of angiogenesis under pathological conditions causes several diseases like diabetic retinopathy, rheumatoid arthritis, and cancer.<sup>2</sup> Thus, the inhibition of tumor angiogenesis provides a therapeutic strategy for treating different types of cancers. In many cases anti-angiogenic agents act as cytostatics and prevent the growth of tumors, though it is thought that they can be made more successful in cancer chemotherapy by combining with cytotoxic agents.<sup>3</sup>

Antioxidants are molecules inhibiting the oxidation of other molecules thereby preventing the cell death that occurs due to the release of free radicals.<sup>4</sup> Reactive oxygen species generated in the cell during anti-oxidation initiate and promote tumor growth as well as other degenerative diseases such as

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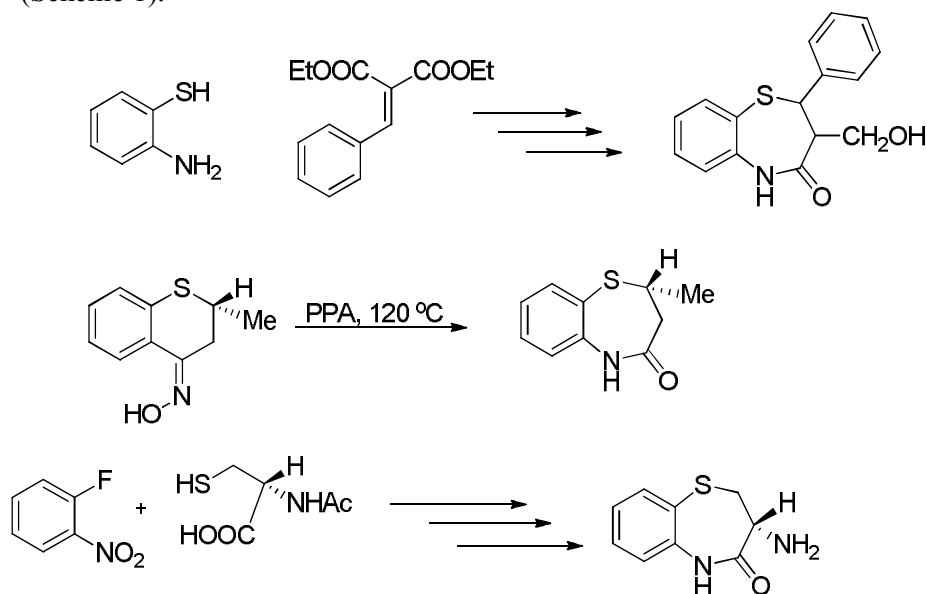
heart attacks, strokes, arthritis and cataracts.<sup>5</sup> Minimizing oxidative damage may be an important approach to the primary treatment of these diseases, since antioxidants prevents the free radical formation, or interrupt an oxidizing chain reaction. Thus, antioxidants can be regarded as important factors for the treatment of cancer. Compounds functioning with groups endowed with potential antioxidant properties are considered as new drugs for chemoprevention and chemotherapy.<sup>6</sup> Therefore, the development of synthetic compounds, capable of scavenging free radicals, has been a great interest.



**Fig. 1.** Examples of 1,5-benzothiazepine drugs

1,5-Benzothiazepines constitute an important class of privileged scaffolds as a result of their immense chemotherapeutic applications, such as calcium channel blockers,<sup>7</sup> angiotensin converting enzyme inhibitors,<sup>8</sup> anticonvulsant,<sup>9</sup> anti-HIV,<sup>10</sup> anti-hypertensive,<sup>11</sup> platelet aggregation inhibitors<sup>12</sup> and cytotoxic<sup>13</sup> agents. They have also been reported as potent antibacterial,<sup>14</sup> antimicrobial,<sup>15</sup> and Bradykinin agonists.<sup>16</sup> Diltiazem [Fig. 1],<sup>17</sup> a well-known angina-relieving calcium channel blocker and coronary vasodilator, and Thiazesim,<sup>18</sup> used as an antidepressant, and the antipsychotic Quetiapine,<sup>19</sup> possess 1,5-benzothiazepine skeletons.

Because of their interesting biological properties, functionalized 1,5-benzothiazepin-4(5H)-ones represent an important class of heterocyclic compounds. The major approaches, for synthesis of 1,5-benzothiazepinones are cyclization and ring expansion. The most widely employed methods involves, nucleophilic attack of substituted 2-aminothiophenols or 2-nitrothiophenols on aliphatic electrophiles<sup>20</sup> and the reaction of 2-fluoronitroarenes with  $\beta$ -mercapto acid, followed by nitro group reduction and cyclisation.<sup>21</sup> (Scheme 1).



**Scheme 1.** Synthetic methods for preparation of 1,5-benzothiazepin-4(5H)-ones

In continuation of our work on development of synthetic methodologies for bioactive molecules,<sup>22,23</sup> we report herein an efficient, versatile and convenient synthetic route which provides rapid access to 1,5-benzothiazepin-4(5H)-ones using a multistep strategy from readily available diethyl di-*n*-butyl malonate **1** and a new coupling agent **T3P** (Figure 2) and their anti-angiogenic and anti-oxidant properties.

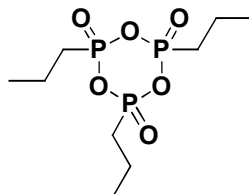
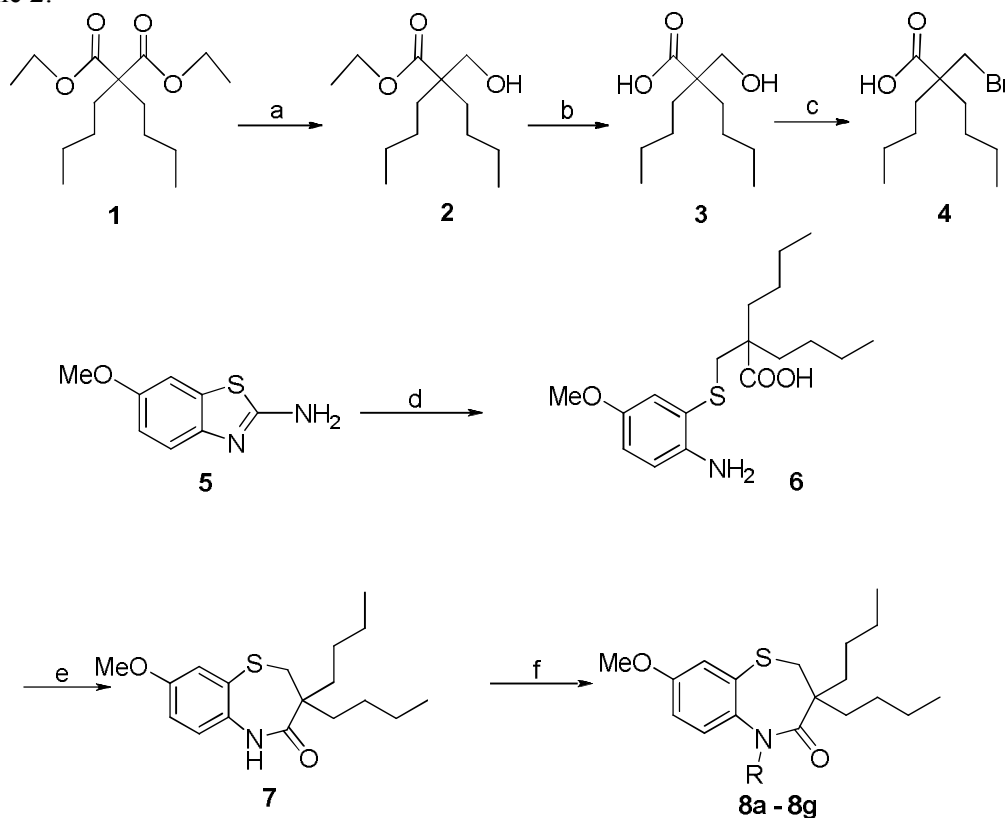


Fig. 2. Structure of T3P

## 2. Results and discussion

### 2.1. Chemistry

The synthesis of 1,5-benzothiazepin-4(5H)-one derivatives was accomplished in seven steps as outlined in Scheme 2.



R = Me	<b>8a</b>
= C <sub>2</sub> H <sub>5</sub>	<b>8b</b>
= allyl	<b>8c</b>
= n-butyl	<b>8d</b>
= isobutyl	<b>8e</b>
= 1-isopropyl urea	<b>8f</b>
= 1-propylpiperidine	<b>8g</b>

(a) DIBAL-H, NaBH<sub>4</sub>; (b) NaOH, MeOH; (c) HBr, conc. H<sub>2</sub>SO<sub>4</sub>; (d) aq. KOH, **4**, THF; (e) T<sub>3</sub>P, Et<sub>3</sub>N; (f) DMF, NaH, RX.

Scheme 2. Synthetic route of 1,5-benzothiazepin-4(5H)-one derivatives

First, ethyl-[2-*n*-butyl-2-hydroxymethyl]hexanoate **2** was obtained by selective reduction of diethyl di-*n*-butyl malonate **1** in toluene using DIBAL-H and NaBH<sub>4</sub> under N<sub>2</sub> atmosphere. Compound ethyl-(2-*n*-butyl-2-hydroxymethyl)hexanoic acid **3** was then synthesized in good yield by hydrolysis of ethyl-(2-*n*-butyl-2-hydroxymethyl)hexanoate **2** in methanol and NaOH. 2-Bromomethyl-2-*n*-butylhexanoic acid **4** was then obtained by treating ethyl-(2-*n*-butyl-2-hydroxymethyl)hexanoic acid **3** with 48% (aq.) HBr and conc. H<sub>2</sub>SO<sub>4</sub>. Amino-6-methoxy benzothiazole **5** in KOH solution was condensed with 2-bromomethyl-2-*n*-butylhexanoic acid **4** in THF under N<sub>2</sub> atmosphere to yield the desired product, 2-[[[(2-amino-5-methoxyphenyl)thio]methyl]-2-*n*-butylhexanoic acid **6**. Finally, the intramolecular cyclization of **6** using T<sub>3</sub>P as cyclodehydrating agent afforded the compound 3,3-di-*n*-butyl-8-methoxy-2,3-dihydro-1,5-benzothiazepin-4(5H)-one **7**. Compounds **8a-8g** were obtained in good yields (85-92%) by alkylation of **7** with different alkyl halides in DMF using NaH as a base under N<sub>2</sub> atmosphere. We concluded, based on previously published work, that this is the first report in which T<sub>3</sub>P is used as a cyclodehydrating agent in the synthesis of the 1,5-benzothiazepin-4(5H)-one core structure **7**. The structures of all the synthesized compounds were confirmed by IR, <sup>1</sup>H NMR and mass spectra.

## 2.2. Antioxidant activities

### 2.2.1. DPPH radical scavenging activity

Antioxidants are characterized by their ability to scavenge free radicals. Proton-radical scavenging action is an important attribute of antioxidants, which is measured by DPPH scavenging assay. DPPH, a protonated radical has significant absorbance maxima at 517 nm which decreases in the presence of antioxidant due to the scavenging of the proton radical<sup>24</sup>. The hydrogen donating ability of the antioxidant molecule contributes to its free radical scavenging potential. The DPPH radical scavenging activity shown by compounds **8a-8g** is due to their H-donating capacity. In the present investigation, compounds **8a-8g** showed low DPPH radical-scavenging activities when compared to BHT (IC<sub>50</sub> values from 136.36 µg/mL to 661.03 µg/mL, BHT has an IC<sub>50</sub> value of 10.87 µg/mL).

### Hydroxyl radical scavenging activity

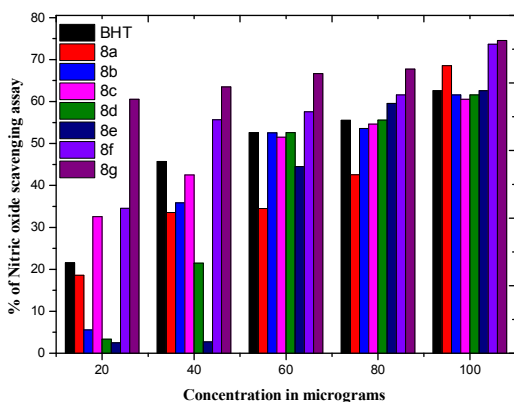
The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology as it is capable of damaging biomolecules found in living cells.<sup>25</sup> Hydroxyl radical has the capacity to cause breakage of DNA, which results in cytotoxicity, carcinogenesis and mutagenesis. In addition, this radical species is rapidly initiates lipid peroxidation, removing hydrogen atoms from unsaturated fatty-acids.<sup>25</sup> In the present study, **8a-8g** showed moderate hydroxyl radical scavenging activity (**8f** with IC<sub>50</sub> value of 13.64 µg and **8b** showing 82.94 µg/mL) when compared to standard BHT (IC<sub>50</sub> value 10.83 µg/mL). The hydrogen-donating capacity of compounds **8a-8g** was observed to be moderate compared to BHT as shown in **Table 1**.

**Table 1.** Free radical scavenging activity of the compounds 8a-8g

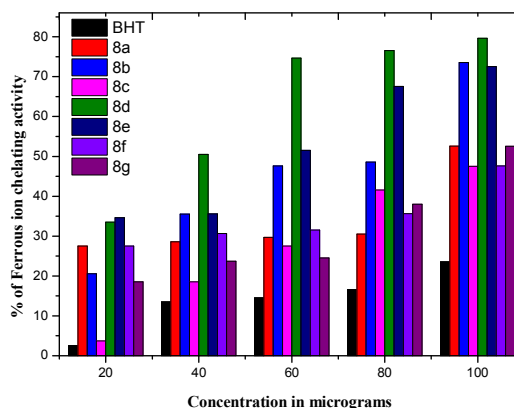
Antioxidant activities				
Compounds	IC <sub>50</sub> values (µg/mL)			
	NO	Fe <sup>2+</sup>	OH	DPPH
8a	85.5	97.91	33.56	136.36
8b	56.40	81.20	82.94	321.43
8c	56.63	105.60	36.47	473.10
8d	58.46	39.59	38.61	661.03
8e	67.42	58.25	30.06	399.61
8f	35.01	104.69	13.64	429.62
8g	16.55	96.38	37.64	301.94
BHT	51.79	213.45	10.83	10.87

### 2.2.2. Nitric oxide radical scavenging activity

Under physiological conditions, nitric oxide (NO) plays important roles as a neurotransmitter, vasodilator and in the immunological system it fights against tumor cells and infectious agents. During inflammatory reactions, NO is produced by the inducible enzyme NO synthase (iNOS) in cells like macrophages, hepatocytes, and renal cells after stimulation by lipopolysaccharide (LPS), tumor necrosis factor (TNF- $\alpha$ ), interleukin (IL-1) or interferon (INF- $\gamma$ ), and acts as a defense and regulatory signal molecule. However, NO is pathogenic when present in excess, as it is a reactive radical itself, and directly damages normal tissues.<sup>26</sup> Further, NO can also react with superoxide anion radical to form the even stronger oxidant, peroxynitrite.<sup>27</sup> Among the samples studied, **8g** and **8f** showed highly significant ( $P < 0.01$ ) activity in scavenging NO radical with  $IC_{50}$  values of 16.55 and 35.01  $\mu\text{g/mL}$ , respectively, when compared to 51.79  $\mu\text{g/mL}$  recorded for BHT (Table 1).



**Fig. 3.** Nitric oxide assay of the synthesized compounds



**Fig. 4.** Ferrous ion assay of the synthesized compounds

### 2.2.3. Ferrous ion chelating ability

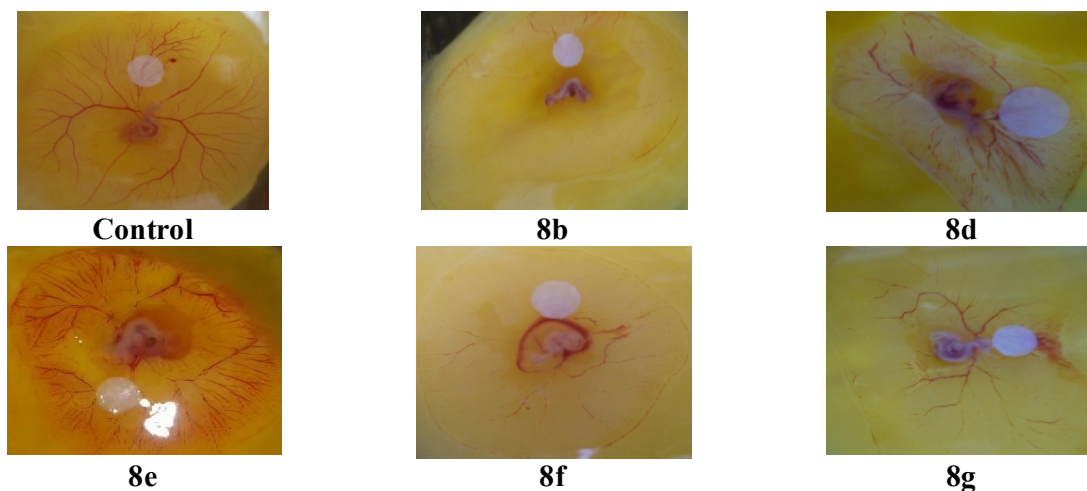
Iron is known to generate free radicals through the Fenton and Haber-Weiss reactions.<sup>28</sup> Metal ion chelating activity of an antioxidant molecule prevents oxy-radical generation and consequent oxidative damage. Metal ion-chelating capacity plays a significant role in anti-oxidant mechanism since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. It is reported that chelating agents, which form sigma-bonds with a metal, are effective as secondary antioxidants because they decrease the redox potential of the metal centre, thereby stabilizing the oxidized form of the metal ion.<sup>29</sup> By virtue of both metal chelating properties and radical scavenging ability, compounds **8a-8g** may have a role in the prevention of free radicals with  $IC_{50}$  values ranging from 39.59  $\mu\text{g/mL}$  for **8d** to 105.60  $\mu\text{g/mL}$  for **8c** (BHT  $IC_{50}$  value = 213.45  $\mu\text{g/mL}$ ).

### 2.3. Antiangiogenic activity

Anti-angiogenic treatment is one of the main methods of tumor treatment and control of pathological angiogenesis.<sup>30</sup> Pathological angiogenesis is regulated by targeting integrins, which are predominantly expressed in most of the tumor cells and endothelial cells of blood vessels. The interaction of vascular endothelial growth factor (VEGF) and its receptors in a signal transduction pathway also regulates angiogenesis.<sup>31</sup>

In the present investigation, some of the compounds among **8a-8g** reduced the proliferation of blood vessels in the shell-less CAM assay model with developing embryos. The proliferation of microvessels

was repressed only in group treated with compounds **8b**, **8d**, **8e**, **8f** and **8g** (Fig. 5) supporting their antiangiogenic activity.



**Fig. 5.** Inhibition of angiogenesis *in vivo* of 1,5-benzothiazepin-4-(5H)-ones (**8a–8g**) in shell-less CAM assay.

### 3. Conclusion

In the present study, novel 1,5-benzothiazepin-4(5H)-one derivatives have been synthesized and their antioxidant and antiangiogenic activities have been assessed. Oxidative stress and angiogenesis are important biological mechanisms by which tumorigenesis and tumor progression occur. Antiangiogenic determined in the shell-less CAM model revealed that **8b**, **8d**, **8e**, **8f** and **8g** compounds are endowed with interesting anti-tumor activities. Further, antioxidants have proved to protect against oncogenic transformations resulting from radiation and free-radicals in experimental systems.<sup>31</sup> The significant multiple antioxidant activities of 1,5-benzothiazepin-4(5H)-one derivatives, especially the high ferrous ion chelating ability of **8a–8g** and nitric oxide radical scavenging activity of **8f** and **8g**, appear to protect the cells by neutralizing or trapping reactive oxygen species and other free radicals. These results conclusively prove that rational design of 1,5-benzothiazepin-4(5H)-one derivatives can be used to create anti-angiogenic and antioxidant agents.

### Acknowledgements

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### 4. Experimental

#### 4.1. General

All reagents were purchased from Sigma Aldrich Chemicals. Infrared (IR) spectra were recorded on a JASCO FT/IR-4100 spectrophotometer in KBr disc for solid compounds and nujol for liquids and are reported in reciprocal wave number ( $\text{cm}^{-1}$ ).  $^1\text{H}$  NMR spectra were recorded in  $\text{DMSO-d}_6$  at 300 and 400 MHz. The following abbreviations are used for structural assignments of  $^1\text{H}$  NMR: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet and br, broad. Mass and purity were recorded on a LC–MSD–Trap–XCT. Thin layer chromatography was performed using 600 mesh silica gel plates, and visualization was effected with short wavelength UV light (254 nm). All other commercial reagents were used as received.

#### 4.2. General procedure for the synthesis of Ethyl-[2-n-butyl-2-hydroxy methyl]hexanoate (2)

Diethyl di-*n*-butyl malonate (25 g, 0.090 mol) was dissolved in toluene (143 mL) under N<sub>2</sub> atmosphere and cooled to -60 to -70 °C. DIBAL-H (25% in toluene, 31.3 g, 0.22 mol) solution was added at this temperature over 15 min, then stirred for 4 h. Absolute ethanol (177 mL) was added slowly at -40 to -50 °C and the temperature was raised to 0 °C. NaBH<sub>4</sub> (3.49 g, 0.090 mol) was added portion-wise below 0 °C and the reaction mixture was stirred at room temperature for 1 h, cooled to 15–20 °C, and saturated Na<sub>2</sub>SO<sub>4</sub> solution was added. The reaction mass was stirred for 1 h, filtered through celite bed, extracted with ethyl acetate (25 mL × 2), washed with brine, dried over sodium sulfate and concentrated under vacuum at 50–55 °C to afford the title compound **2** as a thick syrup.

Pale yellow liquid, yield 19.0 g, 76%; FT-IR (Paraffin)  $\nu/\text{cm}^{-1}$ : 3617, 2857, 1739; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 4.56 (t, 1H, J = 4.8), 4.01 (q, 2H, J = 7.2), 3.42 (d, 2H, J = 5.2), 1.36–1.49 (m, 8H), 1.18 (t, 4H, J = 7.2), 0.80 (t, 6H, J = 8.6); MS (ES) *m/z* (*m*+1): 244.3

#### 4.3. General procedure for the synthesis of Ethyl-[2-n-butyl-2-hydroxy methyl]hexanoic acid (3)

To a stirred solution of ethyl-[2-n-butyl-2-hydroxymethyl]hexanoate (15.0 g, 0.0652 mol) in methanol (67.5 mL), was added NaOH (5.86 g, 0.146 mol) as a solution and the resulting mixture refluxed for 17 h. The methanol was distilled off at 55–60 °C under vacuum, cooled to room temperature and washed with petroleum ether. Aqueous layer was taken back to the reactor, cooled to 10–15 °C and acidified with concentrated HCl to pH 2. The mixture was extracted with dichloromethane (25 mL × 2) and washed with brine solution. The organic layer was dried over sodium sulfate and concentrated under vacuum at 40–45 °C to yield a thick syrup.

Pale yellow liquid, yield 13.73 g, 92%; FT-IR (Paraffin)  $\nu/\text{cm}^{-1}$ : 2857, 3359, 2857; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 11.94 (s, 1H), 3.40 (d, 2H, J = 4.8), 1.34–1.46 (m, 8H), 1.24 (t, 4H, J = 7.2), 0.80 (t, 6H, J = 8.6); MS (ES) *m/z* (*m*+1): 203.3

#### 4.4. General procedure for the synthesis of 2-Bromomethyl-2-n-butylhexanoic acid (4)

To a solution of 48% (aq.) hydrobromic acid was added slowly concentrated H<sub>2</sub>SO<sub>4</sub> below 25 °C over a period of 1 h and then added **2** (10 g, 0.049 mol). The reaction mixture was heated to 90–95 °C and maintained at this temperature for 16 h. The reaction was then cooled to 15–20 °C and extracted with dichloromethane (25 mL × 2). The organic layer was concentrated under vacuum at 40–45 °C, then the residue was distilled using a high vacuum pump. Product distilled at 140–160 °C/1 mm Hg.

Pale yellow liquid, yield 4.36 g, 35%; FT-IR (Paraffin)  $\nu/\text{cm}^{-1}$ : 2857, 2923, 512; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 12.62 (s, 1H), 3.58 (s, 1H), 1.46–1.59 (m, 4H), 1.19–1.28 (m, 8H), 0.80 (t, 6H, J = 8.6); MS (ES) *m/z* (*m*+1): 266.2

#### 4.5. General procedure for the synthesis of 2-[(2-Amino-5-methoxyphenyl)thio]methyl-2-n-butylhexanoic acid (6)

To a stirred solution of amino-6-methoxy benzothiazole (4.00 g, 0.022 mol) in water (44 mL) was added KOH (12.4 g, 0.22 mol). The reaction mixture was refluxed for 16 h under N<sub>2</sub> atmosphere. It was then cooled to 15 °C and added **3** (7.63 g, 0.028 mol) in THF (9.48 mL) slowly over a period of 30 min and then allowed the reaction mass to come to 20–25 °C and stirred for 10 h and then refluxed at 60–65 °C for 2 h. It was then cooled to 20–25 °C and diluted with water. The reaction was then adjusted to pH 5 using 1.5 N HCl, diluted with water and extracted with DCM (25 mL × 2). The organic layer was washed with water and then with brine, dried over sodium sulfate and solvent was removed under reduced pressure to yield a thick syrup.

Dark brown liquid, yield 3.6 g, 90%; FT-IR (Paraffin)  $\nu/\text{cm}^{-1}$ : 3363, 3189, 2923, 2857; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 6.83 (s, 1H), 6.64 (dd, 2H, J = 2.8, 0.4), 3.60 (s, 3H), 2.95 (s, 2H), 1.48–1.56 (m, 4H), 1.13 (t, 4H, J = 6.4), 1.20 (t, 4H, J = 5.6), 0.80 (t, 6H, J = 8.6); MS (ES) *m/z* (*m*+1): 340.3

4.6. *General procedure for the synthesis of 3,3-di-n-butyl-8-methoxy-2,3-dihydro-1,5-benzothiazepin-4-(5H)-one (7):*

To a stirred solution of **4** (3.00 g, 0.0088 mol) in ethyl acetate (27 mL), was added triethylamine (1.95 g, 0.0193 mol). The reaction mixture was cooled to 0 °C and T<sub>3</sub>P (2.81 g, 0.0088 mol) added at 0 °C, then stirred at 25 °C for 3 h and diluted with ethyl acetate. The above organic layer was washed with 10% NaHCO<sub>3</sub> solution, then water, brine, dried over sodium sulfate and concentrated under vacuum at 50 °C to obtain a crude product which was purified by chromatography on silica gel using ethyl acetate and hexane.

Brown solid, yield 3.6 g, 90%; FT-IR (KBr)  $\nu$ /cm<sup>-1</sup>: 3092, 2867, 1213, 1644; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 9.48 (s, 1 H), 7.00 (d, 1 H, J = 8.4), 6.93 (d, 1 H, J = 2.8), 6.82 (q, 1 H, J = 2.8), 3.69 (s, 3 H), 2.94 (s, 2 H), 1.39-1.46 (m, 4 H), 1.14-1.39 (m, 8 H), 0.80 (t, 6 H, J = 8.6); MS (ES) m/z (m+1): 322.0.

4.7. *General procedure for the synthesis of 3,3-Dibutyl-8-methoxy-5-alkyl-2,3-dihydro-5H-benzo[b][1,4]thiazepin-4-one (8)*

A solution of **5** (0.20 g, 0.62 mmol) in DMF (3 mL) was cooled to 0 °C under N<sub>2</sub> atmosphere and NaH (0.0223g, 0.933 mmol) added and stirred for 15 min. To this reaction mixture, methyl iodide (0.0425 mL) was added and the reaction mixture allowed to stir for one hour at room temperature. Progress of the reaction was monitored by TLC. When the reaction was complete, the reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate (25 mL × 2). The organic layer was dried over anhydrous sodium sulfate, and concentrated under vacuum to afford a crude product, which was purified by chromatography on silica gel using ethyl acetate and hexane.

4.7.1. *3,3-Dibutyl-8-methoxy-5-methyl-2,3-dihydro-5H-benzo[b][1,4]thiazepin-4-one (8a)*

Colorless semi-solid, <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.30 (d, 1 H, J = 11.72), 7.10 (d, 1 H, J = 3.84), 7.03 (q, 1 H, J = 7.76, 3.88), 3.75 (s, 3 H), 3.17 (s, 3H), 3.00 (s, 2H), 1.06-1.34 (m, 12 H), 0.78 (t, 3 H, J=8.6); MS (ES) m/z (m+1): 336.4

4.7.2. *3,3-Dibutyl-5-ethyl-8-methoxy-2,3-dihydro-5H-benzo[b][1,4]thiazepin-4-one (8b)*

Colorless semi-solid; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.33 (d, 1 H, J = 8.8), 7.13 (d, 1 H, J = 2.96), 7.03 (q, 1 H, J = 8.8, 3.00), 3.78 (s, 3 H), 3.01 (s, 2 H), 1.00-1.26 (m, 16 H), 0.78 (t, 6 H, J = 8.6); MS (ES) m/z (m+1): 350.4.

4.7.3. *5-Allyl-3,3-dibutyl-8-methoxy-2,3-dihydro-5H-benzo[b][1,4]thiazepin-4-one (8c)*

Colorless semi-solid; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.32 (d, 1 H, J = 8.8), 7.12 (d, 1 H, J = 2.92), 7.04 (q, 1 H, J = 8.8, 2.96), 5.7-5.86 (m, 1 H), 5.14 (d, 1 H, J = 1.36), 5.10 (d, 1 H, J = 1.32), 4.34 (s, 2 H), 3.77 (s, 3 H), 3.03 (s, 2 H), 1.32-1.37 (m, 12 H); MS (ES) m/z (m+1): 362.4.

4.7.4. *3,3,5-Tributyl-8-methoxy-2,3-dihydro-5H-benzo[b][1,4]thiazepin-4-one (8d)*

Colorless semi-solid <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.32 (d, 1 H, J = 8.8), 7.12 (d, 1 H, J = 2.8), 7.03 (q, 1 H, J = 8.8, 2.40), 3.77 (s, 3 H), 3.00 (s, 2 H), 1.16-1.41 (m, 8 H), 1.04-1.16 (m, 9 H), 0.85 (t, 9 H, J = 5.6); MS (ES) m/z (m+1): 378.4.

4.7.5. *3,3-Dibutyl-5-isobutyl-8-methoxy-2,3-dihydro-5H-benzo[b][1,4]thiazepin-4-one (8e)*

Colorless semi-solid <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.34 (d, 1 H, J = 8.8), 7.13 (d, 1 H, J = 2.96), 7.04 (q, 1 H, J = 8.8, 3.0), 3.77 (s, 3 H), 3.04 (s, 2 H), 1.77 (m, 1 H); MS (ES) m/z (m+1): 378.3.

4.7.6. *2-(3,3-Dibutyl-8-methoxy-4-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazepin-5-yl)-N-isopropylacetamide (8f)*

Off-white solid <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 7.66 (d, 1 H, J = 7.2), 7.42 (d, 1 H, J = 8.8), 7.03 (q, 1 H, J = 8.8, 3.2), 3.8 (m, 1 H), 3.76 (s, 3 H), 3.03 (s, 2 H), 1.22-1.34 (m, 14 H), 1.00 (d, 6 H, J = 6.4), 0.77 (t, 6 H, J = 5.6); MS (ES) m/z (m+1): 421.4.



#### 4.7.7. 3,3-Dibutyl-8-methoxy-5(2-piperidin-1-yl-ethyl)2,3-dihydro-5H-benzo[b][1,4]thiazepin-4-one (**8g**)

Yellow semi-solid  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ ):  $\delta = 7.55$  (d, 1 H,  $J = 11.72$ ), 7.08 (d, 1 H,  $J = 3.76$ ), 7.04 (q, 1 H,  $J = 7.8, 3.8$ ), 3.75 (s, 3 H), 2.97 (s, 2 H), 2.25 (m, 4 H), 1.01-1.40 (m, 26 H), 0.77 (t, 6 H,  $J = 5.6$ ); MS (ES)  $m/z$  ( $m+1$ ): 433.4.

#### 4.8. Antioxidant activities

##### 4.8.1. DPPH radical scavenging assay

DPPH radical scavenging activity was carried out according to the method of Scherer *et al.*<sup>32</sup> Briefly, DPPH solution (1 mL, 0.1 mM in 95% ethanol) was mixed with different aliquots of **8a-8g**. After vigorous shaking, the mixture was allowed to stand for 20 min at room temperature. Absorbance of the resulting solution was measured at 517 nm with a UV-VIS spectrophotometer (HITACHI, U-2900). Butylated hydroxyl toluene (BHT) was used as positive control. Radical scavenging potential is expressed as  $\text{IC}_{50}$  value, which represents the sample concentration at which 50 % of DPPH radicals are scavenged.

##### 4.8.2. Hydroxyl radical scavenging assay

The reaction mixture containing different aliquots of **8a-8g**, deoxyribose (10 mM),  $\text{H}_2\text{O}_2$  (10 mM),  $\text{FeCl}_3$  (5 mM), EDTA (1 mM) and ascorbic acid (5 mM) in potassium phosphate buffer (50 mM, pH 7.4) was allowed to stand for 60 min at 37 °C.<sup>33</sup> The reaction was terminated by adding TCA (5% W/V) and the reaction product was measured by reaction with TBA (0.2% W/V) in boiling water bath for 15 min. The absorbance was measured at 535 nm against the reagent blank and inhibition of the oxidation of deoxyribose was calculated against the control using UV-VIS spectrophotometer (HITACHI, U-2900). BHT was used as positive control and radical scavenging potential was expressed as  $\text{IC}_{50}$  value.

##### 4.8.3. Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside in phosphate buffer at physiological pH spontaneously generates nitric oxide, which in turn reacts with oxygen to produce nitrite ions that can be estimated by the Griess reagent.<sup>34</sup> Nitric oxide scavengers compete with oxygen, leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different aliquots of **8a-8g** and incubated at 25 °C for 3 h. The absorbance of the color formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm and referred with the absorbance of BHT treated in the same way with the Griess reagent. The radical scavenging potential was calculated and expressed as  $\text{IC}_{50}$  value.

##### 4.8.4. Ferrous ion chelating ability

Ferrous ion chelating ability was measured according to the method of Suter and Richter.<sup>35</sup> Three sets of test tubes were taken; the first tube as control: To this 40  $\mu\text{L}$  of  $\text{FeCl}_3$  (200  $\mu\text{M}$ ) and 80  $\mu\text{L}$  of  $\text{K}_3\text{Fe}(\text{CN})_6$  (400 mM) were added and the volume was made up to 1 mL by adding distilled water. For the second tube 20  $\mu\text{L}$  of EDTA (400 mM), 40  $\mu\text{L}$  of  $\text{FeCl}_3$  and 80  $\mu\text{L}$   $\text{K}_3\text{Fe}(\text{CN})_6$  were added and the volume was made up to 1 mL by adding distilled water. For the third sample (10  $\mu\text{g}$ -100  $\mu\text{g}$ ), 40  $\mu\text{L}$  of  $\text{FeCl}_3$  and 80  $\mu\text{L}$  of  $\text{K}_3\text{Fe}(\text{CN})_6$  were added and the volume was made up to 1 mL by adding distilled water. The tubes were incubated for 10 min at 20 °C and the OD read at 700 nm. The assay was carried out at 20 °C to prevent  $\text{Fe}^{2+}$  oxidation.

#### 4.9. Antiangiogenic activity

Fertilized eggs were obtained from IVRI, Bangalore, India. All chemicals were purchased from Sisco Research Laboratories, Mumbai, India and they were of analytical grade. Antiangiogenic effects of **8a-8g** compounds was studied according to the method of Auerbach *et al.*<sup>36</sup>. Briefly, fertilized hens eggs were surface sterilized using 70% alcohol. The eggs were incubated in fan assisted humidified incubator at 37 °C. On the 4<sup>th</sup> day, the eggs were cracked out into thin films of the hammock within a laminar flow cabinet and were further incubated. On day 5<sup>th</sup> when blood vessels were seen proliferating from the center of the eggs within the hammock, filter paper discs loaded with 100 µg of the compounds **8a-8g** were placed over the proliferating blood vessels and the eggs were returned to the incubator. Results of antiangiogenic effects of the compounds were noted after 24 h.

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