

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity of novel dihydropyridine derivatives

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ABSTRACT

Thirteen dihydropyridine analogues **1-13** were synthesized and evaluated for their DPPH radical scavenging activity. A good to moderate antioxidant activity ranging from 127.4 to 284.5 μM was observed and structure-activity relationship was established. The 3'-fluoro derivative **8** ($\text{IC}_{50} = 127.4 \pm 3.5 \mu\text{M}$) was found to exhibit highest activity among the dihydro pyridine derivatives **1-13**, while the other derivatives **11** ($\text{IC}_{50} = 132.5 \pm 3.32 \mu\text{M}$), **6** ($\text{IC}_{50} = 142.2 \pm 0.60 \mu\text{M}$), **10** ($\text{IC}_{50} = 144.7 \pm 2.46 \mu\text{M}$), **12** ($\text{IC}_{50} = 153.7 \pm 0.50 \mu\text{M}$), **5** ($\text{IC}_{50} = 161.4 \pm 2.81 \mu\text{M}$) and **5** ($\text{IC}_{50} = 164.4 \pm 2.50 \mu\text{M}$) possess moderate activity, depends upon the C-4 and C-6 substituted groups. The compounds **7**, **13**, **4**, **3** and **2** have lowest IC_{50} values, ranging between 172.8 and 284.5 μM . Dihydropyridine analogues were characterized by spectroscopic techniques.

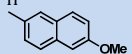
1. Introduction

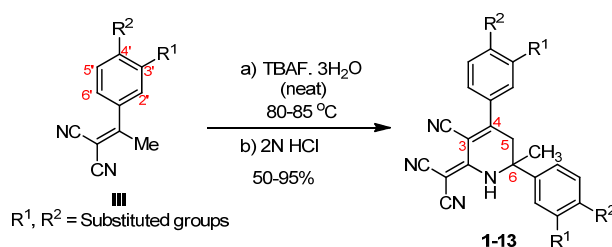
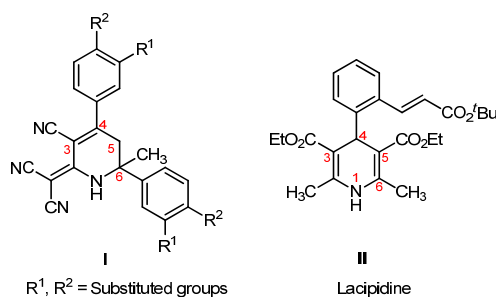
Free radicals, generated during oxidative biochemical processes, can cause many damages to the living systems [1,2]. The over-production of reactive oxygen species (ROS) or weakening of antioxidant defence systems results in oxidative stress which leads to onset of premature aging, cancer and asthma [3-5]. The interaction of free radicals with polyunsaturated fatty acid causes cerebral ischemia and other CNS disorders [6]. Literature showed examples of antioxidants agents bearing aromatic rings, which can inhibit the cellular oxidative damages [7,8]. About 4,000 diverse polyphenols display antioxidant activity *in vitro*, but their antioxidant effects *in vivo* were found to be significantly low [9,10]. Although oxidative reactions are important for physiological function, but the formation of free radicals initiates many undesired reactions in cell, which can lead to cell damage or death. Antioxidants can donate electrons to free radical species and convert them into harmless molecules, and thus prevent cells from oxidative damage [11,12]. Hashida *et al.* demonstrated that the proliferation of metastatic tumor cells can be ceased by the detoxification of hydrogen peroxide with pegylated catalase

[13]. Thus the application of antioxidants to prevent tumor cells progression is an important approach in current drug discovery and developments.

Heterocyclic compounds, particularly the pyridine ring bearing molecules, have a wide range of applications in medicinal chemistry [14]. Cominacino *et al.* [15] reported the antioxidant properties of different 1,4-dihydropyridine derivatives. Lacidipine (II) reduced the concentration of ROS in bovine aortic endothelial cells (BAECs). The antioxidant role of some other types of 1,4-dihydropyridine derivatives has been reviewed by Augustyniak *et al.* [16]. We have recently reported the synthesis of 5,6-dihydropyridine derivatives *via* a solvent free conditions [17]. The antioxidant activity of 1,4-dihydropyridines derivatives encouraged us to evaluate the antioxidant properties of new 5,6-dihydropyridine derivatives (I), having C-4 and C-6 substituted benzene rings (Figure 1). The 5,6-dihydropyridine derivatives were prepared *via* a solvent free method in 52-95 % yields, as shown in Scheme 1. All the synthesized dihydropyridine derivatives (**1-13**) were tested against DPPH which showed a wide range of radical scavenging activity (IC_{50} value from 127.4 to 284.5 μM) due to different substituents on the phenyl ring.

Table 1. *In vitro* DPPH free radical scavenging assay.

Compounds	R ¹	R ²	Yield (%) [17]	IC ₅₀ (μM±SEM ^a)
1	H	H	98	NA ^b
2	H	Me	95	284.5±0.66
3	H	OH	75	228.2±3.36
4	OH	H	93	223.8±3.30
5	H	OMe	95	161.4±2.81
6	OMe	H	80	142.2±0.60
7	H	F	75	172.8±1.72
8	F	H	75	127.4±3.50
9	H	Cl	90	164.6±4.50
10	H	Br	90	144.7±2.46
11	Br	H	90	132.5±3.32
12	H	I	89	153.7±0.50
13			65	196.3±6.5
Ascorbic acid (AS) ^c				40.6±1.2
Butylated hydroxyanisole (BHA) ^c				44.7±0.67

^a SEM is the standard error of the mean.^b NA: Not active.^c Standard inhibitor for DPPH radical scavenging activity.**Scheme 1****Figure 1.** Dihydropyridines I and II.

2. Experimental

2.1. Chemistry

The details of general procedure for the synthesis of dihydropyridine analogues have been published in our previous publication [17]. A schematic layout of the reaction is shown in Scheme 1.

2.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (*in vitro*)

The antioxidant activity of dihydropyridine derivatives against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by following literature protocol [18]. A 5 μL each test sample (0.5 mM in DMSO) was mixed with 95 μL DPPH solution in ethanol. The resulting mixture was incubated for 30 minutes at 37 °C after adding into 96-well microtiter plate. The absorbance was monitored with microplate SpectraMax 340, applied Biosystems, CA, USA, reader at 515 nm. In radical form, DPPH solution in ethanol has strong violet colour, while when react with antioxidant, the colour changed to pale yellow with

decreased in absorbance. The percent radical scavenging activity of each antioxidant was measured in comparison of a DMSO control (Table 1). The IC₅₀ values were calculated by EZ-Fit enzyme kinetics software program (Perrella Scientific Inc. Amherst, MA, USA) (Table 1). Ascorbic acid and butylated hydroxyanisole (BHA) were used as standard inhibitors in DPPH radical scavenging assay.

3. Results and discussion

The synthesis of dihydropyridine derivatives **1-13** is presented Figure 1 [17]. Their structures were deduced by a combination of IR, NMR and mass spectroscopy. Analogues **1-13** were subjected to *in vitro* DPPH radical scavenging assay and results are presented in Table 1.

Dihydropyridine derivatives **1-13** exhibited a varied range of IC₅₀ values 127.4±3.5 to 284.5±0.66 μM (Table 1). The 3'-fluoro **8** (IC₅₀ = 127.4±3.5 μM), 3'-bromo **11** (IC₅₀ = 132.5±3.32 μM), 3'-methoxy **6** (IC₅₀ = 142.2±0.60 μM), 4'-bromo **10** (IC₅₀ = 144.7±2.46 μM), 4'-iodo **12** (IC₅₀ = 153.7±0.50 μM), 4'-methoxy **5** (IC₅₀ = 161.4±2.81 μM) and 3'-chloro **5** (IC₅₀ = 164.4±2.50 μM) derivatives exhibited moderate activity, while 4'-fluoro **7**

($IC_{50} = 172.8 \pm 1.72 \mu M$), **13** ($IC_{50} = 196.3 \pm 6.5 \mu M$), **4** ($IC_{50} = 223.8 \pm 3.30 \mu M$), **3** ($IC_{50} = 228.2 \pm 3.36 \mu M$) and **2** ($IC_{50} = 284.5 \pm 0.66 \mu M$) displayed low activity, when compared to standards inhibitors ascorbic acid ($IC_{50} = 40.6 \pm 1.2 \mu M$) and butylated hydroxyanisole ($IC_{50} = 40.6 \pm 1.2 \mu M$). The phenyl derivative **1** found to be non-active in the series **1-13** (Figure 2).

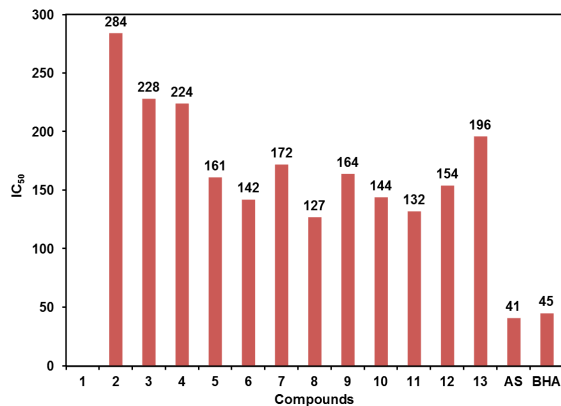


Figure 2. IC_{50} values of dihydropyridine derivatives **1-13**.

Radical scavenging activity of each dihydropyridine derivative (**1-13**) generally depends on the formation and stabilization of resulting free radical species. A structure-activity relationship of dihydropyridine derivatives showed that 3'-fluoro **8** ($IC_{50} = 127.4 \pm 3.5 \mu M$), 3'-bromo **11** ($IC_{50} = 132.5 \pm 3.30 \mu M$) and 3'-methoxy **6** ($IC_{50} = 142.2 \pm 0.60 \mu M$) derivatives exhibited potent activity. This may be due to the formation of free radicals at electron rich centers, such as oxygen atoms. A slight decline in activity was observed in 4'-fluoro **7** ($IC_{50} = 172.8 \pm 1.72 \mu M$), 4'-bromo **10** ($IC_{50} = 144.7 \pm 2.46 \mu M$), 4'-methoxy **5** ($IC_{50} = 161.4 \pm 2.81 \mu M$), 4'-chloro **9** ($IC_{50} = 164.6 \pm 4.50 \mu M$) and 4'-iodo **12** ($IC_{50} = 153.7 \pm 0.50 \mu M$) derivatives, as compared to their 3'-phenyl substituted derivatives **8**, **11** and **6**, possibly due to the *p*-substituted groups of dihydropyridine derivatives.

The hydroxy-substituted analogues **3** (4'-hydroxy) and **4** (3'-hydroxy) showed IC_{50} values $228.2 \pm 30 \mu M$ and $223.8 \pm 30 \mu M$, respectively. Generally, the polyphenolic compounds exhibit good antioxidant activity due to stable phenoxide radical, produced by the abstraction of hydrogen atom by DPPH. However the free radical scavenging activity of our 4'-hydroxy **3** and 3'-hydroxy **4** is found to be lower than the other derivatives in the series. This may be due to stabilization of phenoxide species of these molecules. The DPPH scavenging activity of 4'-methyl derivative **2** ($IC_{50} = 284.5 \pm 0.66 \mu M$) was also found to be low, may be due to electron denoting effect of methyl substituent. This study identifies a new class of compounds with potent DPPH radical scavenging activity. Compound **8** could serve as lead for further development as an antioxidant.

4. Conclusion

The dihydropyridine derivatives **1-13** were identified as a new class of antioxidants. Their IC_{50} values were found to dependent upon the electronic effect of substituents present on the phenyl rings. The 3'-fluoro phenyl analogue **8** was found to be the most active in the series of dihydropyridine derivatives (Table 1). These compounds could serve as lead molecules in the development of new antioxidant with therapeutic potential.

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