SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF SOME AZETIDIN- 2-ONE AS POTENTIAL ANTIFUNGAL AGENTS

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Abstract

A new series of antioxidants, namely azetidinone bearing N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)substituted benzamide (**5a-e**) was designed and synthesized. The synthesized target compounds further confirmed by elemental, IR and NMR spectral studies. Experimental studies of antioxidant activities of the compounds were examined by using DPPH and nitric oxide radical scavenger assays. The DPPH and nitric oxide radical scavenging activities depended on the substitution pattern of the aromatic group. Compound **5d**, which contain a phenolic nitro group at the *para* position exhibited IC_{50} values of 01.12±1.214 µg/ml and potent antioxidant activities against DPPH, which were better than that of the other azetidinone compounds. Similarly compound **5d** showed excellent activity by nitric oxide radical scavenging method. Therefore, azetidinone bearing compound **5d** group can be proposed as potential antioxidants for tackling oxidative stress.

Keywords: 2-azetidinone, antioxidant, DPPH assay and nitric acid assay

1. Introduction

The Reactive oxygen species (ROS) like superoxides ($O_2^{\bullet-}$), alkoxyls ($RO^{\bullet}$), peroxyls ($ROO^{\bullet}$), hydroxyls ($HO^{\bullet}$) and nitric oxides ($NO^{\bullet}$) can direct to cellular injury in the form of damaged DNA, lipids and proteins. Hence, the human body is able to counterbalance ROS by a variety of antioxidant defence mechanisms and immediately eliminate an excess of ROS from the cell by cellular antioxidant enzymes and other redox molecules [1]. Natural and synthetic antioxidants may protect against cells damage by virtue of their ability to scavenge free radicals. Scientists from various disciplines are interested in new antioxidants compounds of both synthetic and natural origin, to prevent or reduce the impact of oxidative stress on cells [2,3]. Various compounds have been designed and chemically synthesized based on data for the structural requirements of potent antioxidants. These compounds are only required in minute amounts. Generally, the free radical scavenging capacity of phenols is attributed to the hydrogen atom on the hydroxyl group [4], while nitrogen and sulphur analogues may provide alternatives for labile hydrogen atoms [5-7].

Azetidinone are well-known compounds with a wide range of pharmacological activities, including anti-inflammatory, antiviral, antimicrobial, anticonvulsant, antitumor, fungicidal activities and antihistaminic [8-14]. As azetidinone derivatives rarely exist in nature, probably, due to the difficulty in the construction of the N-N bond of living organisms, their availability mainly depends on the synthetic methods [15]. They have also shown potent antioxidant activities with regards to scavenging free radicals [16,17]. In this study we aimed to generate a series of azetidinone as prodrugs. A series of substituted benzhydrazide comprising diverse electron-withdrawing and electron attracting groups were chosen, so that the impact of substitution on the free radical releasing pattern and the antioxidant activity could be further discussed. After being synthesized and characterized, the molecules

antioxidative properties were evaluated using DPPH and nitric oxide radical scavenging methods

2. Experimental

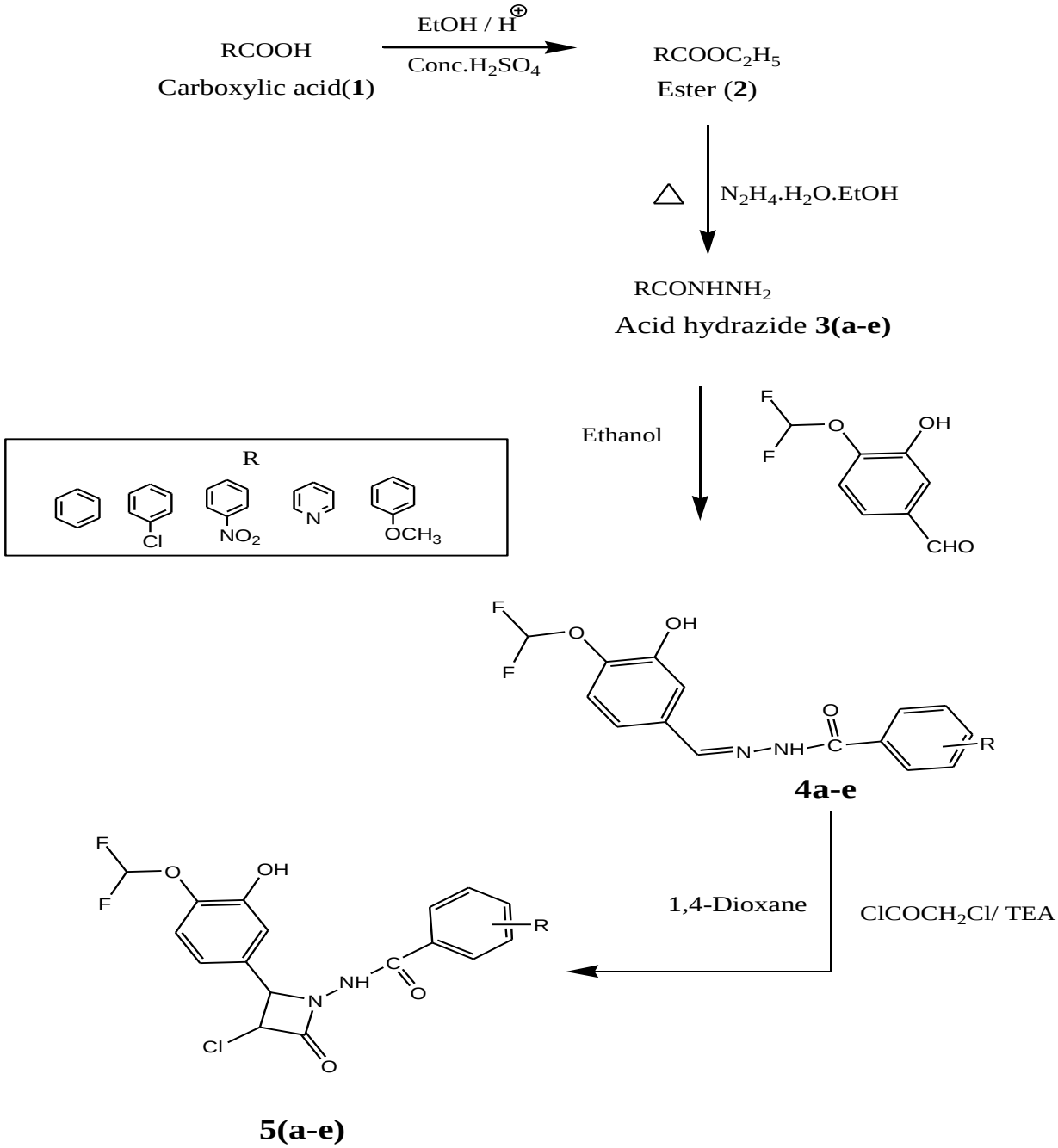
2.1.Materials and methods.

All the chemicals and solvents used were of AR grade obtained from Sigma Aldrich, Lobachemie (India). Melting points of the synthesized compounds were determined in open-glass on a Staurt-SMP10 melting point apparatus and recorded in ˚0 C without correction. The purity of the compounds was ascertained by thin layer chromatography on silica gel coated aluminum plates (Merck) as adsorbent and UV light as visualizing agent. Synthesized compounds were recrystallised using ethanol as solvent. IR spectra were recorded on SHIMADZU FT-IR spectrometer using KBr pellet technique. ^{1}H-NMR spectra were recorded on BRUKER-400 spectrometer operating at 400 MH_Z using TMS as internal standard in DMSO (chemical shifts in ppm).

3. Results and discussions

3.1. General procedure for the synthesis of compounds 3a-e: In a 250 ml round bottom flask, a mixture of substituted carboxylic acid (0.1mol), ethanol (60 ml) and conc. H_2SO_4 (1.4 ml) were refluxed for 10 hours on a water bath. The solution was cooled and poured slowly with stirring on to 200 g of crushed ice. Sufficient ammonia solution was added to render the resulting solution alkaline, generally some ester separates as oil but most of it remains dissolved in the alkaline solution. The solution was extracted five times with ether (25 ml) the combined ethereal extract was dried with anhydrous $MgSO_4$. Ether was removed by evaporation on a water bath and the residue was collected. Physical data of ester was noted. The Synthetic procedure is shown in scheme 1.

3.2 General procedure for the synthesis of compounds 4a-e[27]: A mixture of ester and hydrazine hydrate in 1:1 portion and ethanol (30 ml) were taken in a round bottom flask and refluxed for 4-6 hrs. Excess of ethanol was removed by distillation. On cooling the product, acid hydrazide separates out. It was filtered and collected. Recrystallization was carried out with ethanol and physical data was noted. The physical properties of synthesized derivatives 3(a-e) are shown in Table 1.



Scheme I: Synthesis of the 2-Azetidinone derivatives 5(a-e)

3.3 Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)benzohydrazide (4a)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{15}H_{12}F_2N_2O_3$; M.Wt:306; yield 67%, mp 142-146 ^{0}C; IR (KBr, m, cm $^{-1}$): 2908 (-Ali CH), 3030 (-Aro CH), 1562 (-CH=N), 1687 (-C=O), 3197 (-OH) and 1172 (-C-F) ;1HNMR: (DMSO-d6, 400 MHz), δ 8.37 (s, 1H, CH=N), δ 7.12 (s, 1H, -NH), δ 6.93 (s, 1H, -OH), δ 7.30 (s, 1H, -CHF_2) and δ 7.12-7.92 (m, 8H, Aromatic protons)

3.4 Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)-4-chlorobenzohydrazide (4b)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{15}H_{11}ClF_2N_2O_3$; M.Wt:340; yield 72%, mp 115-117 ^{0}C; IR (KBr, m, cm $^{-1}$): 2960 (-Ali CH), 3134(-Aro CH), 1598 (-CH=N), 1637 (-C=O), 3317 (-OH), 1109 (-C-F) and 750 (-C-Cl);1HNMR: (DMSO-d6, 400 MHz), δ 8.30 (s, 1H, CH=N), δ 7.00 (s, 1H, -NH), δ 6.89 (s, 1H, -OH), δ 7.19 (s, 1H, -CHF_2) and δ 7.07-7.90 (m, 7H, Aromatic protons)

3.5 Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)-4 nitrobenzohydrazide (4c)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{15}H_{11}F_2N_3O_5$; M.Wt:351; yield 65%, mp 110-112 ^{0}C; IR (KBr, m, cm $^{-1}$): 2931 (-Ali CH), 3062 (-Aro CH), 1589 (-CH=N), 1687 (-C=O), 3304 (-OH) and 1128 (-C-F; 1HNMR: (DMSO-d6, 400 MHz), δ 8.36 (s, 1H, CH=N), δ 7.04 (s, 1H, -NH), δ 6.94 (s, 1H, -OH), δ 7.23 (s, 1H, -CHF_2) and δ 7.04-8.15 (m, 7H, Aromatic protons)

3.6 Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)isonicotinohydrazide (4d)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{14}H_{11}F_2N_3O_3$; M.Wt:307; yield 68%, mp 124-126 ^{0}C; IR (KBr, m, cm $^{-1}$): 2912 (-Ali CH), 3035 (-Aro CH), 1568 (-CH=N), 1645 (-C=O), 3288 (-OH) and 1170 (-C-F) ;1HNMR: (DMSO-d6, 400 MHz), δ 8.34 (s, 1H, CH=N), δ 7.11 (s, 1H, -NH), δ 6.93 (s, 1H, -OH), δ 7.30 (s, 1H, -CHF_2) and δ 7.14-8.77 (m, 7H, Aromatic protons)

3.7 Synthesis of N'-(4-(difluoromethoxy)-3-hydroxybenzylidene)-4-methoxybenzohydrazide (4e)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{16}H_{14}F_2N_2O_4$; M.Wt:336; yield 75%, mp 135-137 ^{0}C; IR (KBr, m, cm $^{-1}$): 2931 (-Ali CH), 3053 (-Aro CH), 1591 (-CH=N), 1687 (-C=O), 3329 (-OH) and 1130 (-C-F) ;1HNMR: (DMSO-d6, 400 MHz), δ 8.33 (s, 1H, CH=N), δ 7.05 (s, 1H, -NH), δ 6.91 (s, 1H, -OH), δ 7.42 (s, 1H, -CHF_2), δ 3.83 (s, 3H, -OCH_3) and δ 7.04-7.91 (m, 7H, Aromatic protons)

3.8 General procedure for the synthesis of compounds (5a–e)

Hydrazones 3a-f (0.04 mol) and triethylamine (0.02 mol) in dioxan (20 mL) at 0–5^{o}C mixture was stirred for 5 h. During stirring, chloroacetyl chloride (0.01 mol) in dioxan (10 mL) was added dropwise. The mixture was refluxed for 2 h and kept for two days on room temperature. The resulting mixture was poured in the water and the solid was separated out. Recrystallization was done with ethanol–water or chloroform– water to give the azetidine-2-one, 4a-f compounds.

Table 2: Physical properties and IR Spectral Data of Synthesized Hydrazides 3(a-e)

Compound	R	Molecular formula	M.Pt (˚C)	M.Formula	Physical state	IR Frequency
3a	C_6H_5	$C_7H_8N_2O$	110	136	White powder	3032 cm^{-1} (C-H Ar str); 1622 cm^{-1}(C=O str); 3296, 3215 ($NHNH_2$ str); 985 cm^{-1} (N-N str).
3b	C_6H_5Cl	$C_7H_7ClN_2O$	162	170	White powder	3016 cm^{-1} (C-H Ar str); 1645 cm^{-1} (C=O str); 3302, 3209 cm^{-1} ($NHNH_2$ str); 727 cm^{-1} (C-Cl str); 987 cm^{-1} (N-N str).
3c	$C_6H_5NO_2$	$C_7H_7N_3O_3$	215	181	Pale Yellow powder	3070 cm^{-1} (C-H Ar str); 1662 cm^{-1} (C=O str); 3296, 3180 cm^{-1} ($NHNH_2$ str); 1525 cm^{-1} (NO_2 str); 1055 cm^{-1} (N-N str).
3d	C_5H_5N	$C_6H_7N_3O$	170	137	White powder	3064 cm^{-1} (C-H Ar str); 1649 cm^{-1} (C=O str); 3305, 3178 cm^{-1} ($NHNH_2$ str); 1045 cm^{-1} (N-N str).
3e	$C_6H_5OCH_3$	$C_8H_{10}N_2O_2$	135	166	White powder	3043 cm^{-1} (C-H Ar str); 2933 cm^{-1} (C-H Aliphatic str); 1612 cm^{-1} (C=O str); 3381, 3253 cm^{-1} ($NHNH_2$ str); 1031 cm^{-1} (N-N str).

3.8.1 Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)benzamide (5a)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{17}H_{13}ClF_2N_2O_4$; M.Wt:382; yield 64%, mp 208-210 ^{0}C; IR (KBr, m, cm^{-1}): 2926 (-Ali CH), 3051 (-Aro CH), 1681 (-C=O), 3425(-OH); 1H NMR (400 MHz, DMSO-d6) (ppm): 4.83(d, 1H, –CH-N), 9.73 (d, 1H, OH), 4.53 (d, 1H, –CH-Cl) 7.09-8.04 (m, 4H, Ar-H)

3.8.2 Synthesis of 4-chloro-N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)benzamide (5b)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{17}H_{12}C_{l2}F_2N_2O_4$; M.Wt:417; yield : 60%, mp 235-237 ^{0}C; IR (KBr, m, cm $^{-1}$): 2920 (-Ali CH), 3047 (-Aro CH), 1664 (-C=O), 3435 (-OH); 1H NMR (400 MHz, DMSO-d6) (ppm): 4.62(d, 1H, –CH-N), 9.89(s, 1H, OH), 4.48(d, 1H, –CH-Cl) 6.86-7.82(m, 4H, Ar-H)

3.8.3 Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-nitrobenzamide (5c)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{17}H_{12}ClF_2N_3O_6$; M.Wt:427; yield : 56%, mp 240-242 ^{0}C; IR (KBr, m, cm $^{-1}$): 2926 (-Ali CH), 3051 (-Aro CH), 1664 (-C=O), 3431 (-OH); 1H NMR (400 MHz, DMSO-d6) (ppm): 4.37(d, 1H, –CH-N), 11.89 (s, 1H, OH), 4.14 (d, 1H, –CH-Cl) 7.16-8.35 (m, 4H, Ar-H)

3.8.4 Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)isonicotinamide (5d)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{16}H_{12}ClF_2N_3O_4$; M.Wt:383; yield : 65%, mp 210-212 ^{0}C; IR (KBr, m, cm $^{-1}$): 2924 (-Ali CH), 3045 (-Aro CH), 1674 (-C=O), 3425 (-OH); 1H NMR (400 MHz, DMSO-d6) (ppm): 4.77 (d, 1H, –CH-N), 11.84 (s, 1H, OH), 4.72 (d, 1H, –CH-Cl) 6.56-8.35 (m, 4H, Ar-H)

3.8.5 Synthesis of N-(3-chloro-2-(4-(difluoromethoxy)-3-hydroxyphenyl)-4-oxoazetidin-1-yl)-4-methoxybenzamide (5e)

This was prepared and purified as per the above mentioned procedure: M.F: $C_{18}H_{15}ClF_2N_2O_5$; M.Wt:412; yield : 60%, mp 182-184 ^{0}C; IR (KBr, m, cm $^{-1}$): 2927 (-Ali

CH), 3043 (-Aro CH), 1672 (-C=O), 3425 (-OH); 1H NMR (400 MHz, DMSO-d6) (ppm): 4.78(d, 1H, –CH-N), 11.69 (s, 1H, OH), 4.69 (d, 1H, –CH-Cl) 6.91-7.91 (m, 4H, Ar-H)

3.9 Analysis of the antioxidant activities

3.9.1. DPPH radical scavenging activity

The DPPH free radical scavenging assay was performed according to the method of Brand-Williams [18] with slight modifications. The reaction mixture was prepared by mixing 195 ml of a 100 μM methanolic DPPH solution with 50 ml of the azetidinone compounds at different concentrations (500, 250, 125, 62.5 and 31.25 μg/mL). The test compounds were initially dissolved in dimethyl sulfoxide (DMSO). After 30 min of incubation in the dark at room temperature, the absorbance of the reaction mixture was determined at 515 nm. The colour of the reaction mixture changed from purple to yellow as a result of the decreased absorbance. The radical scavenging activity was calculated by the following equation:

$$\text{Scavenging activity (\%)} = \frac{A0 - A1}{A0} \times 100$$

where A0 is the absorbance of the DPPH radical without a sample or standard; and A1 is the absorbance of the DPPH radical with a sample or standard. IC50 values which represent the efficient concentration of the samples that inhibit 50% of the DPPH radicals were calculated and expressed in mg/mL [19-21].

3.10. Nitric oxide free radical scavenging activity

The procedure is based on the principle that, sodium nitro-prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Large amounts of NO• may lead to tissue damage. 500, 250, 125, 62.5 and 31.25 μg/mL of each of the

concentrations of azetidinone compounds previously dissolved in DMSO. To each tube 2.0 ml of sodium nitroprusside (10 μM) in phosphate buffer saline was added. The solutions were incubated at room temperature for 150 minutes. The similar procedure was repeated with methanol as blank which served as control. After the incubation, 5 ml of griess reagent was added to each tube including control [22-24].

$$\text{Scavenging activity } (\%) = \frac{[Abosrbance\ control - Absorbance\ of\ test\ sample]}{Absorbance\ of\ control} \times 100$$

4 Results and discussion

4.1.DPPH free radical scavenging activity

One of the quick methods to evaluate antioxidant activity is the scavenging activity on DPPH, a stable free radical and widely used index. The DPPH radical scavenging ability strongly depends on the geometric accessibility of the radical trapping site. The presence of steric hindrance may prevent a test compound from reaching the radical site of DPPH, resulting in a low activity. The DPPH assay is based on either a hydrogen atom transfer (HAT) or a single electron transfer (SET) mechanism. In the DPPH free radical scavenging activity, azetidinone **5a-e** was evaluated for their free radical scavenging activity. The IC_{50} was calculated for each azetidinone and summarized in **Table 1.**

Table 2 DPPH free radical scavenging activity for azetidinone compounds

	Percentage of scavening ± SD				
Concentration /Entry	**5a**	**5b**	**5c**	**5d**	**5e**
500	13.75±0.242	14.71±0.257	58.38±1.722	68.17±0.611	71.34±0.510
250	07.17±0.311	08.24±1.215	51.10±1.293	32.53±0.220	65.14±1.150
125	04.25±1.210	05.14±0.0201	54.50±0.844	26.53±1.220	61.65±0.071
62.5	02.45±1.210	03.41±0.111	33.30±1.317	09.45±0.100	51.55±1.417
31.5	04.45±1.210	15.28±1.123	01.12±1.214	02.95±1.110	01.34±1.170

Preliminary antioxidant evaluation was conducted at 500, 250, 125, 62.5 and 31.25 µg/ml test concentration. It can be seen, from **Table 2**, compounds **5a-e** present the highest scavenging activity at 71.34 µg/ml concentration. The compound **5d** present the highest scavenging activity (68.17 µg/ml) on DPPH radical, whereas the **5c** present moderate and **5b** and **5a** present very low scavenging activity on DPPH radical. The activity of synthesized compounds is remarkably higher than that of naturally occurring glycosmicine 2 (21.7 ± 0.38 lg/mL). The main reason might be that the free methoxy or pyridine group is at the *para* position. The wide variations in free radical scavenging activities may be due to the variations in the proton–electron transfer by the compounds due to difference in their structures and stability. The presence of methoxy groups on the phenyl ring greatly influenced antioxidant activity as observed for all of the compounds of this study. Along with all derivatives, eight azetidinone showed promising effect in DPPH scavenging property which follow the order; **5e**>**5d**>**5c**>**5b**>**5a**.

4.2.**Nitric oxide scavenging activity**

Nitric oxide scavenging activity was performed with designed compounds and the results are tabulated in **Table 3**. Antioxidant activity was examined at different concentration namely 500, 250, 125, 62.5 and 31.25 µg/ml. Eight bioactive compounds (**5a-e**) were found to exhibit strong inhibitory activity giving at 500µg/ml concentration. The compound **5d and 5c** present the higher scavenging activity, whereas the **5b and 5e** present moderate nitic oxide scavenging activity. Meanwhile, chloro and methoxy group derived derivative displayed moderate inhibitory activity. Rest of compounds showed lower activity. From structure activity relationship, it can be seen in **Table 3** that antioxidant activity of derivatives **5d**, showed the higher free radical scavenging activity due to attachment of pyridine moiety in which may be involved in free radical mechanism. Literature survey also shows that nicotinic

group is responsible for antioxidant activity [25]. From structure activity relationship, it is clear that nicotinic group is essential for antioxidant activity. Besides, it has also been found that the substitution at *para* position as methoxy (**5e**) does effect the nitric oxide activity due to strong effect of electron donating moiety. As seen from **table 3** , five azetidinone showed promising effect in nitric oxide scavenging property which follow the order; **5d>5c>5b>5e>5a.**

Table 2 Nitric oxide free radical scavenging activity for azetidinone compounds

	Percentage of scavening ± SD				
Concentration /Entry	**5a**	**5b**	**5c**	**5d**	**5e**
500	31.15±1.222	57.18±0.711	67.65±0.827	73.33±0.321	56.40±0.743
250	25.14±1.111	41.07±1.311	52.20±1.627	64.23±0.312	50.20±1.427
125	14.62±0.152	32.43±1.201	50.30±1.427	58.13±0.123	42.53±0.210
62.5	09.17±0.066	22.18±1.122	42.51±0.120	34.53±0.114	33.33±0.221
31.5	4.14±0.125	10.51±0.120	03.14±0.045	11.75±0.123	03.33±0.123

5. Conclusion

The newly synthesized analogues were evaluated for their in vitro antioxidant activity. Antioxidant results exhibited that derivatives **5d and 5e** showed much better activity than other compounds. Our current finding suggested that methoxy and pyridine substituent is a crucial moiety for antioxidant activity and might have promising therapeutic potential as antioxidant agents. Results of antioxidant activity demonstrated that nitro substituted azetidinone derivatives exhibited significant antioxidant activity.

References

[1] K. Apel, H. Hirt, Annu. Rev. Plant Biol. 55 (2004) 373.
[2] N. Dharmaraj, P. Viswanathamurthi, K. Natarajan, Transit. Met.Chem. 26 (2001) 105.
[3] A. Corona-Bustamante, J.M. Viveros-Paredes, A. Flores-Parra, A.L. PerazaCampos, F.J. Martínez-Martínez, M.T. Sumaya-Martínez, A. Ramos-Organillo, Molecules 15 (2010) 5445.
[4] E.G. Bakalbassis, A.T. Lithoxoidou, A.P. Vafiadis, J. Phys. Chem. A 110 (2006) 11151.
[5] L.E.S. Netto, H.Z. Chae, S.W. Kang, S.G. Rhee, E.R. Stadtman, J. Biol. Chem. 271 (1996) 15315 .
[6] S. Raweh, I. Bayach, M. Taha, M.S. Baharudin, F. Di Meo, M.H. Hasan, A. Adam, N.H. Ismail, J.-F.F. Weber, P. Trouillas, J. Comput. Aided Mol. Des. 27 (2013) 951.
[7] A. Padmaja, C. Rajasekhar, A. Muralikrishna, V. Padmavathi, Eur. J. Med. Chem. 46 (2011) 5034.
[8] A.K. Tewari, A. Mishra, A, Bioorg. Med. Chem. 9 (2001) 715.
[9] S.L. Janus , A.Z. Magdif, B.P. Erik, Monatsh. Chem. 130 (1999)1167.
[10] E.V. Pimerova, E.V. Voronina, Pharm Chem J 35 (2001) 602.
[11] I. Bouabdallah, L.A. Barret, A. Zyad, A. Ramadan, I. Zidane, A. Melhaoui, Nat. Prod. Res. 20(2006)1024.
[12] H.J. Park, K. Lee, P.J.Park, B. Ahn, J.C. Lee, H.Y. Cho, K.I. Lee, Bioorg. Med. Chem. Lett. 15 (2005) 3307.
[13] C. K. Chu, J.J. Cutler, Heterocycl. Chem. 23 (1986) 289.
[14] V. Michon, C.H. Du Penhoat, F. Tombret, J.M. Gillardin, F. Lepagez, L. Berthon, Eur. J. Med. Chem. 30 (1995)147.
[15] V. Kumar, K. Kaur, G.K. Gupta, A.K. Sharma, Eur. J. Med. Chem. 69 (2013)735.
[16] A. Padmaja, C. Rajasekhar, A. Muralikrishna, V. Padmavathi, European Eur. J. Med. Chem. 46 (2011) 5034.
[17] A. Padmaja, T. Payani, G. Dinneswara Reddy, V. Padmavathi, Eur. J. Med. Chem. 44, (2009) 4557.
[18] W. Brand-Williams, M. Cuvelier, C. Berset, LWT-Food Sci. Technol. 28 (1995) 25.

[19] Y. Ünver, K. Sancak, F. Celik, E. Birinci, M. Küçük, S. Soylu, N.A. Burnaz, Eur. J. Med. Chem. 84 (2014) 639.
[20] G. Wang, Y. Xue, L. An, Y. Zheng, Y. Dou, L. Zhang, Y. Liu, Food Chem. 171 (2015) 89.
[21] S. Shenvi, K. Kumar, K.S. Hatti, K. Rijesh, L. Diwakar, G.C. Reddy, Eur. J. Med. Chem. 62 (2013) 435.
[22] .K. Jain, R.K. Agrawal, Asian J. Exp. Sci. 22 (2008) 213.
[23] A.E. Mohammad, M.N. Seyed, F.N. Seyed, F. Bahramian, A.R. Bekhradnia, Pak. J. Pharm. Sci. 23 (2010) 29.
[24] Lahvale, S. Manish, S.H. Mishra, Indian J. Exp. Bio. 45 (2007) 376.
[25] K. I. Priyadarsini, D. K. Maity, G. H. Naik, M. S. Kumar, M. K. Unnikrishnan, J. G. Satav, H. Mohan, Free Rad. Biol. Med. 35 (2003) 475.