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Synthesis, characterization and free radical scavenging activity of 2 azetidinone Derivatives

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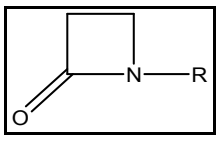
Abstract: Some new derivatives of 2-azetidinone were synthesized by the reaction of N-substituted-1, 3, 4-oxadiazol-2-yl)-3-chloro-4-phenylazetidin-2-one **(4a-4e)** by refluxing substituted (styryl)-N-benzylidene-1,3,4-oxadiazol-2-amine in GAA, which is synthesized by substituted cinnamic acid with urea in the presence of $POCl_3$. The synthesized derivatives were evaluated for free radical scavenging activity. The results of pharmacological activity indicate that compounds **4c** exhibited more activity among synthesized compounds at various concentration.
Keywords: Free radical scavenging activity, DMSO, 2-azetidinone derivatives.

Introduction

Pharmaceutical chemistry are disciplines at the intersection of chemistry, especially synthetic organic chemistry, pharmacology and various other biological specialties, where they are involved with design, chemical synthesis and development for market of pharmaceutical agents or bio-active molecules (drugs). The primary objective is to synthesize and develops new compounds for different biological uses. Azetidinone contains a β-lactam (cyclic amide), four member rings. It is a well known heterocyclic compound among the organic and medicinal chemists. This β-lactam is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. β-lactam compounds are the most commonly used antibiotics. It is the simplest β-lactam compound. The first synthetic β-lactam was prepared by Hermann Staudinger in 1907 by reaction of the Schiff base of aniline and benzaldehyde with diphenylketone in a [2+2] cyclo addition. Azetidinone can be prepared from Schiff's bases, which are the condensation products of aldehyde and amino compounds. They are considered significant owing to their wide range of biological applications. They are also employed as intermediates in chemical synthesis.



Azetidin-2-one is a hydrolytically sensitive colorless, solid, M.P 73-74^{o}C other simple azetidin-2-ones are usually low melting solids or oils.

Free radicals (FRs) are naturally formed in a wide range of biological as well as chemical systems. They are chemical stable atoms and molecules, which have one (or rarely more) free electron/electrons in the electron envelope [1-3]. Almost all biomolecules, but mainly biomembranes, proteins and nucleic acids, may be attacked by reactive free radicals. Free radicals are responsible for many pathological processes, or they can be

generated as the result of the pathological stage and cause important secondary damage to biological systems and cells [1,4-7]. Connections between free radicals and some serious diseases, including Parkinson´s and Alzheimer´s disease, atherosclerosis, heart attacks, and chronic fatigue syndrome, have been demonstrated. However, short-term oxidative stress (OS), the unbalance between the formation and scavenging of the reactive oxygen species, may be important in the prevention of aging due to the triggering the process known as mitohormesis [6,8-13]. On the average, 65 – 70 % of the population is excessively impacted by oxidative stress caused by FRs. Therefore, OS monitoring is an important part of reasonable health prevention [4, 8, 9, 14-18

The protective system of the organisms is based on the activity of specific enzymes (especially superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase) as well as non-enzymatic compounds with antioxidant activity (α-tocopherol, L-ascorbic acid, glutathione, coenzyme Q10, flavonoids, albumin and other still unidentified molecules) called antioxidants [1,8,10-12,15,19-22]. This intricately linked system provides a hydrogen radical, which is able to react with the reactive free radical forming a neutral compound [14,23]. The antioxidant activity is one of the ways how Methods of the antioxidant activity determination are usually based on the direct reaction of the studied molecule with radicals (scavenging) or on the reaction with transition metals.

Free radicals and reactive oxygen species (ROS) including super oxide anion, hydrogen peroxide, and hydroxyl radical are being generated during normal cellular metabolism and bioorganic redox process. Furthermore, radical reactions play a significant role in the development of life-limiting chronic diseases such as cancer, hypertension, cardiac infarction, stroke, arteriosclerosis, rheumatoid arthritis, Alzheimer's and Parkinson diseases, cataracts, and others [1-4]. Exposure of a normal cell to free radical is known to damage structures and consequently interfere, with functions of enzymes and critical macromolecules (e.g., lipids, proteins and nucleic acids).

The human body possesses innate defense mechanisms to counter free radicals in the form of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The unbalance between formation and detoxification of free radical species results in the progression of oxidative stress and leads to the development of chronic and degenerative diseases. Therefore, inhibition of oxidative damage by supplementation with an antioxidant and/or free radical scavengers might reduce the risk of these diseases [5, 6]. In the past decade, medicinal chemists, food chemists, and biologists have increasingly focused their attention on researching and testing for new and efficient natural or synthetic antioxidants as a protective strategy against these diseases by reducing and/or inhibiting free radical reactions.

2-azetidinones, commonly known as β-lactams, are well-known heterocyclic compounds among organic and medicinal chemists [24]. The activities of famous antibiotics penicillins, aztreonam, and carbapenems are attributed to the presence of 2-azetidinone ring in their structure.

Thiazole derivatives display a wide range of biological activities such as antimicrobial [29], anticancer [30], antitubercular [31], antihelmintic [32], and diuretic [33]. Antimicrobial activities of some substituted thiazoles are well established because they possess (S–C=N) toxophoric unit. Thiazoles have enhanced lipid solubility with hydrophilicity, easily metabolized by routine biochemical reactions and noncarcinogenic in nature [34].

Emerging infectious diseases and increasing number of multidrug-resistant microbial pathogens still make the treatment of infectious diseases an important and pressing global problem. Therefore, a substantial research for the discovery and synthesis of new classes of free radical scavenging activity are needed [35, 36].

Tuberculosis (TB) remains among the world's great public health challenges. Worldwide resurgence of TB is due to the two major problems: the acquired immunodeficiency syndrome (AIDS) epidemic, which started in the mid-1980s, and outbreak of multi drug-resistant tuberculosis (MDR-TB). Thus, there is an urgent need for anti-TB drugs with improved properties such as enhanced activity against MDR strain, reduced toxicity, shortened duration of therapy, rapid mycobactericidal mechanism of action, and the ability to penetrate host cells and exert anti mycobacterial effects in the intracellular environment. As a result, there is a pressing need for new antitubercular agents acting with greater potency and efficacy than the existing drugs [37].

Moreover, indole, thiazole, azetidinone, and thiazolidinone are well famed for their broad spectrum of biological activities. In the light of the above reports and in continuation of our research on the synthesis of bioactive indole derivatives [38–41], a drug strategy has been planned to synthesize indole derivatives containing thiazole, azetidinone, and thiazolidinone moieties with the hope to get improved biological activities.

Experimental Procedure:

1. Synthesis of Cinnamic acid derivatives

(0.2 mol) of benzaldehyde derivatives,(0.29 mol) of acetic anhydride and (0.122mol) of freshly fused and finely powdered potassium acetate in a dry, 250 ml round bottomed flask and fitted with in air condenser carrying a calcium chloride guard tube. Mix well and heat the reaction mixture in a oil bath at 160 ^{0}C for 1 hr. Pour the mixture while still hot (80-100 ^{0}C) into 100 ml of water container in a litre rbf which has previously been fitted for steam distillation, . Now add aqueous solution of sodium carbonate until a drop of the liquid withdrawn on the end of a glass rod turns red litmus a distinct blue. Acidify the filtrate by adding conc. Hcl slowly and with vigorous stirring until the evolution of the CO_2. Recrystillised from 3:1 of water and rectified spirit .The yield of dry cinnamic acid was obtained.

2. General procedure for the synthesis of 1, 3, 4-oxadiazole

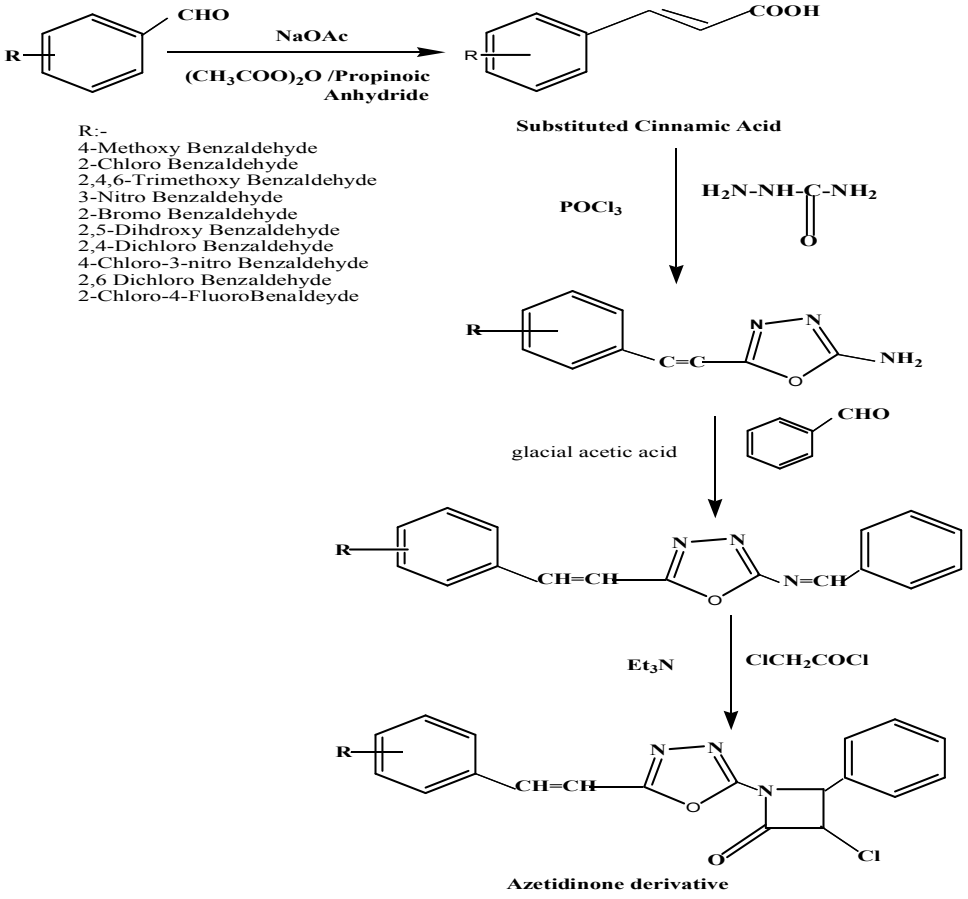
To a solution of compound (step1) in ethanol (25ml), Semicarbazide Hydrochloride (0.2mol) was added and refluxed for 8 hrs in the presence of potassium hydroxide and the resulting solid mass poured into ice water, filtered, washed with water and then recrystillised from absolute ethanol.

3. General procedure for the synthesis of Schiff bases

To a mixture (step 2) of (0.005 mol) and substituted aromatic aldehyde (0.005 mol) in absolute ethanol (40ml) was added few drops of glacial acetic acid. The mixture was refluxed on water bath for 5-6hrs. The excess of solvent was distilled off, poured onto ice cold water. The separated solid was filtered, washed and recrystillised from ethanol**.**

4. General procedure for the synthesis of 2-azetidinone derivatives (4a-4e)

Chloroacetylchloride (0.01) ml was added drop wise to Schiff's base(step3) and triethylamine (0.02ml) in dioxane (25ml) at 5-10^{o}C. The mixture was stirred for 20 hours and left at room temperature for three days. The content were filtered, dried and recrystillised from ethanol.



(4a-4e)

[4a] 1-(5-(2-chlorostyryl)-1, 3, 4-oxadiazol-2-yl)-3-chloro-4-phenylazetidin-2-one

IR (KBrcm^{-1}) (Ar-CH)-3070, (N-N)-1765, (C=N)-1689, (C-Cl)-765, (C=O Azetidinone ring)-1734, (C=C) -aliphatic & aromatic-1668 &1678, (C-O-C)-1123, (N=CH)-1674, (C-H)-2890; **^{1}H NMR(δ)in ppm** (1H, s, CH-Cl of Azetidinone ring) 4.1, (1H, s, Azetidinone proton) 3.1, (10H, m, Ar-H) 7.2 to 7.5, (HC=CH) 5.8, (NH) 1.8, (1H, s, CONH) 8.3

Mass (m/z): 374; **Elementary analysis**: C-59.08, H-3.39, Cl-18.36, N-10.88, O- 8.28

[4b] 1-(5-(4-bromostyryl)-1, 3, 4-oxadiazol-2-yl)-3-chloro-4-phenylazetidin-2-one

IR (KBr cm^{-1}) (Ar-CH)-3026,(N-N)-1865, (C=N)-1689,(C-Br)-765, (C=O Azetidinone ring)-1776, (C=C)-aliphatic & aromatic-1634&1639, (C-O-C)-1167,(N=CH)-1756,(C-Cl)-654,(C-H)-2978.(C-Cl)-623; **^{1}H NMR(δ)in ppm** (1H, s, CH-Cl of Azetidinone ring) 4.2, (1H, s, Azetidinone proton) 3.2, (10H, m, Ar-H) 7.1 to 7.3, (HC=CH) 4.8, (NH) 1.4, (1H, s, CONH) 8.1; **Mass (m/z):** 428.45; **Elementary analysis**:- C-52.99, H-3.04, Br-18.55, Cl-8.23, N-9.76,O-7.4.

[4c]1-(5-(2-hydroxy-5-nitrostyryl)-1,3,4-oxadiazol-2-yl)-3-chloro-4-phenylazetidin-2 one: IR (KBr cm-^{1}) (Ar-CH)-2908, (N-N)-1689, (C=N)-1646, (OH)-3654, (C=O Azetidinone ring)-1730, (C=C)-aliphatic & aromatic-1689 & 1725, (C-O-C)-1240, (N=CH)-1765, (N=O)-1534, (C-H)-2983, (C-Cl)-764**; ^{1}H NMR (δ)in ppm** (1H, s, CH-Cl of Azetidinone ring) 4.2, (1H, s, Azetidinone proton) 3.2, (10H, m, Ar-H) 7.1 to 7.3, (HC=CH) 4.8, (NH) 1.4, (1H, s, CONH) 8.1**; Mass (m/z):** 378; **Elementary analysis**:- C 55.28, H 3.17, Cl 18.36, N 10.88, O 8.28

[4d] 1-(5-(2-methoxystyryl)-1, 3, 4-oxadiazol-2-yl)-3-chloro-4 phenylazetidin-2-one

IR (KBrcm^{-1}) (Ar-CH)-3098, (N-N)-1587, (C=N)-1678, (C=O)-1702, (C=O Azetidinone ring)-1778, (C=C)-aliphatic & aromatic-1623 &1545, (C-O-C)-1123, (N=CH)-1765, (C-Cl)-765, (C-H)-2987; **^{1}H NMR (δ) in ppm** (1H, s, CH-Cl of Azetidinone ring) 4.5, (1H, s, Azetidinone proton) 3.2, (10H, m, Ar-H) 7.5, (HC=CH) 3.6, (NH) 1.2, (1H, s, CONH) 8.0; **Mass (m/z):** 379; **Elementary analysis**:- C-62.91, H-4.22, Cl-9.29, N-11.01, O 12.57.

[4e] 1-(5-(2-bromo-4-hydroxystyryl)-1,3,4-oxadiazol-2-yl)-3-chloro-4-phenylazetidin-2-one: IR (KBrcm^{-1}) (Ar-CH)-3025, (N-N)-1768, (C=N)-1650,(C-Br)-745, (C=O Azetidinone ring)-1715, (C=C) -aliphatic & aromatic-1623&1698, (C-O-C) 1145, (N=CH)-1567, (C-Cl)-780, (C-H)-2897, (OH)-3605; **^{1}H NMR (δ) in ppm** (1H, s, CH-Cl of Azetidinone ring) 3.9, (1H, s, Azetidinone proton) 3.1, (10H, m, Ar-H) 7.3, (HC=CH) 4.8, (NH) 1.3, (1H, s, CONH) 8.4; **Mass (m/z):** 440; **Elementary analysis**: - C 51.09, H 2.93, Br 17.89, Cl 7.94, N 9.41,O 10.75

Characterization of data of Compound

Compound	R	Molecular formula	M.P (^{0}C)	Yield %	Recrystillised Solvent
4a	**2-chloro**	$C_{19}H_{13}Cl_2N_3O_2$	205-209^{0}C	55%	Ethanol
4b	**4-bromo**	$C_{19}H_{13}BrClN_3O_2$	280-285^{0}C	80%	Ethanol
4c	**2-hydroxy-5-nitro**	$C_{19}H_{13}Cl_2N_3O_2$	198-204^{0}C	78%	Ethanol
4d	**2-Methoxy**	$C_{20}H_{16}ClN_3O_3$	210-215^{0}C	45%	Ethanol
4e	**2-Bromo-4-hydroxy**	$C_{19}H_{13}BrClN_3O_2$	215-219^{0}C	76%	Ethanol

Antioxidant Activity 10-13:

All the synthesized derivatives (4a-4e) were tested for their in-vitro free radical scavenging Nitric oxide (NO) and scavenging of Superoxide radical with the alkaline DMSO method. Assay of nitric oxide radical scavenging activity14: Nitric oxide radical scavenging activity was assayed by using Griess reagent. The reaction mixture contained 5 ml of sample solution and ascorbic acid (standard) of different concentrations (25-100 µg / ml) in standard phosphate buffer solution (pH 7.4) , 5ml of sodium nitroprusside solution (5 mM) in

standard phosphate buffer (pH 7.4) and incubated for 5 hours at 25°C. Control was prepared without compound but with an equivalent amount of buffer. Then 0.5ml of the incubation mixture was mixed with 0.5 ml of Griess reagent (Sulphanilamide 1%, o-phosphoric acid 2% and naphthyl ethylene diamine dihydro chloride 0.1%) and the absorbance was measured at 546 nm against blank (DMSO). The experiments were performed in triplicate. From the absorbance the percent of scavenging activity was calculated as follows and the results were shown in Table 1

$$\text{Scavenging activity (\%)} = \frac{[(A\ (control)-A\ (sample))]}{A\ (control)} \times 100$$

Assay of Superoxide Radical Scavenging Activity[15]:

Superoxide radical scavenging activity was assayed by nitro blue tetrazolium system. The reaction mixture containing 0.1ml of nitro blue tetrazolium (1mg/ml in DMSO) and 0.3ml of synthesized compounds (4a-4e) or standard in DMSO was added (1ml of DMSO containing sodium hydroxide 5mM in 0.1 ml of water) to give a final volume of 1.4ml and the absorbance was measured at 560nm against blank (DMSO). The percentage scavenging of super oxide radical was calculated by using above formula. The results were shown in Table 2

Table 1: Nitric oxide Radical Scavenging activity for 2-azetidinone derivatives (4a-4e)

S.No	Conc.(mcg/ml	Scavenging activity (%)						
		Std.	**Control**	**4a**	**4b**	**4c**	**4d**	**4e**
1	**25**	23.06	6.01	11.63	09.01	12.68	07.63	10.14
2	**50**	34.63	6.01	24.89	07.44	22.32	17.07	16.56
3	**75**	45.68	6.01	33.76	28.45	39.80	29.01	27.99
4	**100**	59.32	6.01	50.97	36.88	49.91	35.99	43.14

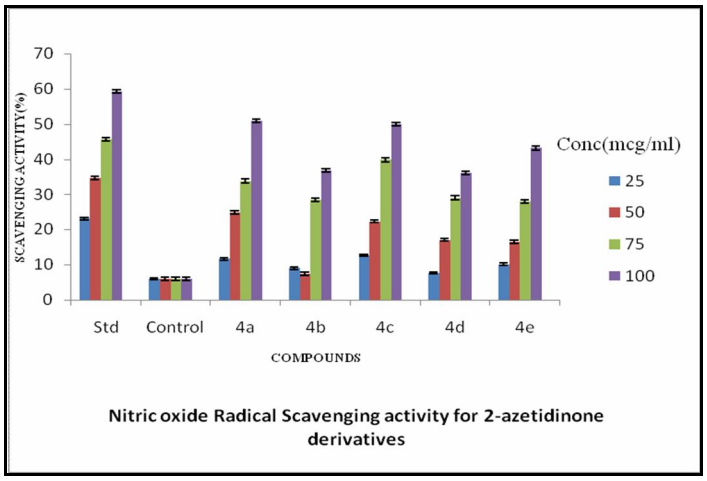
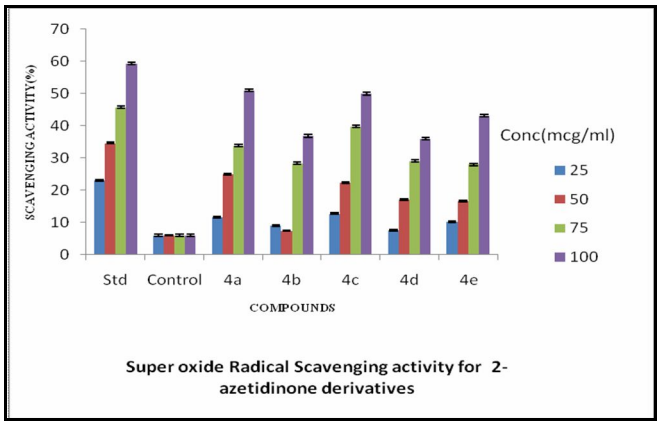


Table 2: Super oxide Radical Scavenging activity for 2-azetidinone derivatives (4a-4e)

S. No	Conc. (mcg/ml)	Scavenging activity (%)						
		Std.	**Control**	**4a**	**4b**	**4c**	**4d**	**4e**
1	**25**	22.98	6.99	07.67	06.34	12.02	10.08	06.26
2	**50**	34.04	6.99	10.67	14.93	29.86	20.08	22.65
3	**75**	44.17	6.99	26.76	29.49	33.89	28.99	24.65
4	**100**	60.01	6.99	54.97	39.98	50.01	30.70	28.06



Super oxide Radical Scavenging activity for 2-azetidinone derivatives

Results and Discussion:

The nitric oxide assay method has been widely used to evaluate the free radical scavenging activity of various derivatives. Nitric oxide generated as a result of decomposition of sodium nitroprusside in aqueous medium, interacts with oxygen at physiological pH to produce nitrite ions, which were measured by using Griess reagent. The nitrite ions were subjected to diazotization followed by azo-coupling reaction to yield an azo-dye, measured by an absorption band at 546nm. The scavenging ability of the synthesized compounds was compared with ascorbic acid as a standard. Compounds 4c produced better scavenging ability (Table-1). Compounds 4a 4d showed moderate radical scavenging activity. Even though superoxide radical is a weak oxidant, it gives rise to the generation of powerful and dangerous hydroxyl radical along with single oxygen; both radical lead to oxidative stress. The experimental results suggest that 4c showed better scavenging activity whereas 4a, 4b, 4d & 4e exhibited moderate activity.

Conclusions

2-azetidinone derivatives exhibited significant to moderate activity when compared with standard ascorbic acid. Strong free radical scavenging activity was observed for **4c** in both methods. The antioxidant capacity of the compounds were found to be 06.26 to 54.97 for different concentrations (25-100 µg / ml)

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