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Pharmacological Evaluation of Aldehydic-Pyrrolidinedione Against HCT-116, MDA-MB231, NIH/3T3, MCF-7 Cancer Cell Lines, Antioxidant and Enzyme Inhibition Studies

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Purpose: The current work was designed to synthesize a bioactive derivative of succinimide and evaluate it for anti-Alzheimer, anticancer and anti-diabetic potentials.

Methods: The compound was synthesized by Michael addition of butyraldehyde with *N*-phenylmaleimide. The synthesized compound was screened for biological potentials including anti-cholinesterase, in-vitro anti-diabetic, antioxidant and anthelmintic potentials. The anti-cholinesterase potential was evaluated against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), anti-diabetic potential against α-glucosidase, antioxidant potential against ABTS, DPPH and H_2O_2 and anthelmintic potential against *Perethima posthuma* and *Ascaridia galli* respectively.

Results: The compound demonstrated significant AChE and BChE inhibition i.e., 71.34 ±1.92 and 73.42 ±1.92 at the concentration of 1000 µg/mL respectively. Other dilutions exhibited concentration-dependent inhibitory activity against both enzymes. In the MTT assay, the newly synthesized compound was found active against all of the cell lines viz, HCT-116, MDA-MB231, NIH/3T3 and MCF-7 and the highest cytotoxicity potential was observed against the colon cancer cell line (HCT-116) with an IC_{50} value of 78 µg/mL exhibiting its highest potential. Moreover, the compound exhibited prominent α-glucosidase inhibitory potentials (79.86±2.54% at 1000 µg/mL) with IC_{50} value of 156.23 µg/mL. Further, our test compound exhibited considerable scavenging activity against DPPH, ABTS and H_2O_2 free radicals with percent inhibitions of 75.84±1.58, 72.85±1.17 and 54.82±1.82 and IC_{50} values of 84.36, 139.74 and 752.21 µg/mL respectively. Our test sample exhibited significant anthelmintic potentials. It demonstrated significant paralysis and death of the test worms in an unbelievably short time in comparison with albendazole.

Conclusion: Going into the detail of all observations, it may be deduced that the newly synthesized succinimide derivative could be an important drug candidate against neurodegenerative disorders like Alzheimer's disease, cancer, diabetes mellitus and worms. Further detailed studies in animal models are required for in-vivo analysis of the compound.

Keywords: Succinimide, Alzheimer's disease, MTT, oxidative stress, diabetes, helminthiasis

Introduction

The world has long been combating various types of health crises in different ways. It has been evidenced that the world has overcome the majority of health challenges but still there is dire need for continuous research activities to facilitate life with good health.[1] Pharmacotherapy is playing a vital role in the management of various

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types of health anomalies. The drug has been provided by nature in the form of natural products or in the form of raw materials from which we synthesize a variety of synthetic drugs.[2–4] Some of the raw materials include a special type of scaffold which is proved to be very effective after some modification, by formation of derivatives via addition of different functional groups. The synthetic derivatives have been proved to provide some extraordinary drug candidates to the market to treat various disease conditions. Some diseases are still there which target specific groups of people and are not curable although they could be managed with the help of medicinal products. For instance, Alzheimer's disease (AD) is one of the diseases which target the old age population and a variety of drug candidates are used for the management of the symptoms related to AD.[5–8] AD is prevalent in those countries which have a large number of old age population, which comprise developed countries of the world.[9] Similarly, the underdeveloped nations of the world are facing different malnutrition problems due to the absence of proper food and proper hygienic measures.[10–12] Inappropriate hygienic conditions also lead to the infestation of individuals by different types of worms.[13–15] Worm infestation has been reported to be the cause of a large number of gastrointestinal, liver and blood problems.[16] In the same way, free radicals have been reported to be the cause of different challenging diseases including cancer.[17–20] Among all of the challenging diseases, the most lethal and horrible is obviously cancer, which could only be cured if properly diagnosed in the first two stages but still the drugs exploited against the cancer are associated with a plethora of adverse effects. Moreover, one of the disease conditions which are prevalent around the globe is diabetes mellitus. This disease has also been managed successfully with the help of different types of drugs.[21,22]

Valuable scaffolds of the drug type molecules have been synthesized and explored by organic and medicinal chemists.[23–26] As stated earlier, synthetic drugs have added a lot to the management and therapy of different types of diseases. One of the scaffolds which have been showing promising results in the formation of bioactive derivatives is the succinimide. Different succinimide derivatives have been synthesized and demonstrated with significant biological activities.[27–29] Based on the clinical importance of succinimide and the need of the day, the current research project has been designed to synthesize a bioactive derivative of succinimide and evaluate its biological potentials against cholinesterases, cancer cell lines, α-glucosidase, worms and free radicals.

Materials and Methods

Chemicals

For synthesis of the aldehyde derivative of succinimide, all chemicals, reagents and solvents which were used in this research work include *N*-phenylmaleimide (CAS No. 941-69-5), butyraldehyde (CAS No. 123-72-8), potassium hydroxide (CAS No. 1310-58-3), dichloromethane (DCM) (CAS No. 75-09-2), ethyl acetate (EtOAc) (CAS No. 141-78-6), *n*-hexane (CAS No. 110-54-3), methanol (CAS No. 67-56-1) and dimethyl sulfoxide (DMSO) (CAS No. 67-68-5) purchased from Sigma-Aldrich GmbH USA. The reaction progress was observed using analytical chromatographic plates (TLC Silica gel 60 F_{254}) made in Germany purchased from the native market in Peshawar, KPK, Pakistan.

For *in-vitro* inhibition of cholinesterase, the chemicals including AChE from electric eel (type-VI-S, CAS No. 9000-81-1) and BChE from equine serum lyophilized (CAS No. 9001-08-5) were purchased from Sigma-Aldrich GmbH USA. Acetylthiocholine iodide (CAS No. 1866-15-5) and butyrylthiocholine iodide (CAS No. 2494-56-6) which stand in as enzyme substrates were purchased from authorized Sigma-Aldrich Switzerland and Sigma-Aldrich UK respectively. The indicator substance for cholinesterase inhibition assay, 5,5-dithio-bis-nitrobenzoic acid (DTNB) (CAS No. 69-78-3), was purchased from Sigma-Aldrich Germany. Galanthamine hydrobromide Lycoris Sp. (CAS No. 1953-04-4), acting as a standard drug, was purchased from Sigma-Aldrich France.

The intended assay for free radical scavenging, the chemicals and reagents, with DPPH (CAS No. 1898-66-4, Sigma Aldrich Chemie GmbH USA), ABTS (CAS No. 30931-67-0, Sigma Aldrich USA), $K_2S_2O_4$ (Riedel-de Haen Germany) and gallic acid (CAS No. 149-91-7, GmbH USA), and hydrogen peroxide (CAS No. 7722-84-1) were acquired from Merck Co. (Germany). The cell lines were purchased from ATCC (http://www.lgcstandards-atcc.org/) and the studies were conducted in Dr. Panjwani Centre for Molecular Medicine and Drug Research, ICCBS, University of Karachi, Pakistan.

Synthesis of 2-(2,5-Dioxo-1-Phenylpyrrolidin-3-Yl)Butanal

Michael addition was used for C–C bond formation between the Michael acceptor and donor.[30] To promote the reaction,

L-isoleucine (0.1 mol%, 13.117 mg) and potassium hydroxide (0.1 mol%, 5.6 mg) were added to a small reaction vessel containing 1.0 mL of DCM (dichloromethane). Then, butyraldehyde (2.0 mmol, 0.359 µL) and 1.0 mmol (173.17 mg) of *N*-phenylmaleimide were added at room temperature as shown in Scheme 1. Subsequently, TLCs (EtOAc/*n*-hexane 20:80) were taken. After 10 h, the reaction was fully completed. Thereafter, the reaction was diluted with 15 mL of water. Extraction was done with 15 mL of dichloromethane in triplicate. Organic layers combined were dried with Na_2SO_4, filtered and finally concentrated via rotary evaporator. The resultant mixture was further purified using column chromatography.[29]

Anticancer Studies

MTT Assay

The synthesized compound was investigated against cancer cell lines viz, NIH/3T3, HCT-116, MDA-MB-231, MCF-7 and a normal cell line i.e., WI-38 at various concentrations via 96-well flat microplate reader at a wavelength of 570 nm following the standard MTT assay.[31] Briefly, the selected cell lines were cultured in DMEM medium, supplemented with 10% of FBS along with selected antibiotics i.e., 100 µg/mL of streptomycin and penicillin (100 IU/mL) in flasks and incubated at 37 °C containing 5% of CO_2. Cell cultures were prepared having an initial density of 6×10^4 cells/mL with 80% confluence and 200 µL/well of media was transferred into 96-well plates, which were put in an incubator. The medium was discarded from each plate after overnight incubation and the same amount of fresh medium with different concentrations (31.25–1000 ppm) of test samples was added into each well. After 48 h, 200 µL MTT (0.5 mg/mL) was transferred into each well and put in an incubator for 4 h. Afterward, DMSO (100 µL) was transferred into each microplate well. The extent of reduction of MTT to formazan within the cells was calculated by measuring the absorption value at 570 nm, through a microplate reader (Spectra Max plus; Molecular Devices, CA, USA). The IC_{50} value was recorded for the samples causing 50% cytotoxicity for all of the cell lines.

Anti-Cholinesterase Studies

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory assays were performed following our previously reported spectrophotometric method.[6] In this assay, both enzymes act on their respective substrates to form an intermediate compound called 5-thio-2-nitrobenzoate anion. This anion forms a complex with another reagent called DTNB and result in the yellow color compound formation which is quantified on UV.

Preparation of Solutions

Our test sample was dissolved in previously prepared 0.1 M phosphate buffer to form solutions ranging from 62.5 to 1000 µg/mL. For the adjustment of the solution pH, potassium hydroxide was used. For the preparation of AChE solution 518 U/mg and for BChE solution 7–16 U/mg was dissolved in phosphate buffer to form 0.03 U/mL and 0.01 U/mL solutions respectively.[27] Likewise, DTNB solutions (0.0002273 M), acetyl thiocholine iodide (ATchI) and butyryl thiocholine iodide (BTchI) 0.0005 M solutions were made using double-distilled water. All solutions were maintained in Eppendorf tubes in the refrigerator. Standard drug (galanthamine) solutions were prepared in methanol at the same concentrations.

Spectroscopic Analysis

In the spectroscopic analysis, solutions of the enzymes (5 µL) and 205 µL of test compound were sequentially added to the cuvette. Thereafter, 5 µL of DTNB solution was added to the mixture in the cuvette. The mixture of three reagents was sustained for 15 min at 30 °C using a water

Scheme 1 Michael addition of butyraldehyde to *N*-phenylmaleimide.

bath. Finally, 5 μL of substrate solution was added to the reaction mixture. Absorbance was recorded via UV spectrophotometer at 412 nm. A solution of the standard drug (galanthamine 10 μg/mL) worked as the control drug whereas all solutions except the test compound were considered the negative control. UV absorbance values beside the reaction time were recorded for 4 min at 30 °C. All assays were performed three times and inhibitory values for enzymes were obtained as follows:

$$V = \Delta Abs/\Delta t$$

$$\%\ \text{enzyme inhibition} = 100 - \%\ \text{enzyme activity}$$

$$\%\ \text{enzyme activity} = V/V_{max} \times 100$$

where Vmax is activity of the enzyme in the complete absence of inhibitor agent.

α-Glucosidase Inhibition Studies

The anti-α-glucosidase potential of the synthesized compound was carried out with the help of a chromogenic assay.[32,33] For such assay, α-glucosidase solution was made having composition of 0.5 unit/mL and 20 μL of prepared solution was mixed with 120 μL of phosphate buffer (pH 6.9). For the substrate, *p*-nitrophenyl-α-D-glucopyranoside solution (5mM) was prepared in the same buffer. Briefly, 10 μL of test sample having various concentrations (31.25–1000 μg/mL) was added to them in a test tube and kept back for 15 min at 37 °C. After incubation, 20 μL of already prepared substrate solution was added to all of the samples and incubated for a further 15 min. To terminate the reaction, sodium carbonate solution (0.2 M) having a volume of 80 μL was transferred to all tubes. Absorption at 405 nm was recorded for each sample. The reaction mixture with the test sample served as the control while acarbose served as the positive control. The % enzyme inhibitory potential was determined as follows:

Antioxidant Studies

DPPH Radical Scavenging Assay

The standard procedure was followed for the anti-radicals properties of the sample against DPPH free radicals.[34] The stock solutions of synthesized compound were made in different dilutions (62.50–1000μg/mL). To start the assay, 0.1 mL from each dilution of our synthesized compound was transferred to 3.0 mL of already prepared DPPH solution in methanol. The mixture was incubated for 30 min and absorbance values were recorded for all groups of samples at 517 nm via UV spectrophotometer. The % scavenging effect of samples was calculated using the formula: $[(A_0–A_1)/A_0]\times100$. Here, A_0 is control group absorbance and A_1 represents our test compound absorbance.

ABTS Anti-Radicals Study

The ABTS anti-radicals assay was used following our previously reported procedures.[35,36] In brief, various solutions including $K_2S_2O_4$ and ABTS (7 mM) were prepared and combined. The mixture was incubated (25 °C) in a dark place for about 12–16 h to generate sufficient ABTS radicals. The resultant ABTS solution was diluted using previously prepared 0.01 M phosphate buffer (pH 7.4) and the absorbance of the solution was adjusted to an absorbance value of 0.70 at 734 nm.

Later, 300 μL of our compound solution was mixed with 3.0 mL of ABTS radicals solution in an UV cuvette and the decrease in the absorbance was recorded. Like the DPPH assay, ascorbic acid again acted as the reference drug. Anti-radicals potentials were calculated as:

H_2O_2 Anti-Radicals Study

To assess further the anti-radicals potentials of our synthesized compound, an H_2O_2 assay was performed.[37] A 2 mM H_2O_2 solution prepared in 50 mM phosphate buffer (pH 7.4) was mixed with the previously prepared solution (0.1 mL) of test samples of various concentrations. UV absorbance was recorded at 230 nm against the blank after 10 min and the H_2O_2 scavenging effect was calculated as:

Anthelmintic Studies

Anthelmintic potentials of our test compound were evaluated against *Pheretima posthuma* and *Ascaridia galli* at concentrations of 10–40 mg/mL.[14,38] Due to both physiological and anatomical similarity with human intestinal roundworm *Ascaris lumbricoides*, *P. posthuma* were selected for investigation. The selected *P. posthuma* were collected on searching a muddy soil of Swabi, KPK, Pakistan, having average length of about 7–9 cm. *A. galli* were collected from the intestine of domestic chickens. Previously prepared different concentrations of the test compound (10 mg, 20 mg and 40 mg) in distilled water and Tween 80 by making a suspension of them, 25 mL of each, were transferred into sterilized Petri dishes (150×15 mm). An aqueous solution of albendazole was also prepared in the same concentration. From each solution, 25 mL was transferred to a Petri dish followed by six worms into each Petri dish with the help of forceps. Paralysis and death times were observed in hot water at 50 °C.

Results

Chemistry

The compound 2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)butanal was synthesized in a single-step reaction and a shorter time of 10 h at room temperature. The isolated yield of the compound was 85%. The Rf value of the synthesized compound was 0.46. The ^{1}H NMR and ^{13}C NMR spectra are shown in Figures 1 and 2 respectively. ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 1.14 (t, *J*=7.50 Hz, 3H), 1.67–1.76 (m, 1H), 1.92–2.02 (m, 1H), 2.51 (dd, *J*=18.42, 5.11 Hz, 1H), 2.92–3.05 (m, 2H), 3.29–3.41 (m, 1H), 7.26–7.38 (m, 5H), 9.76 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) (ppm): 12.72, 19.93, 31.98, 39.01, 53.48, 127.94, 128.63, 128.72, 128.78, 135.72, 175.37, 177.47 and 202.92.

Biological Studies

Results of MTT Assay

The results of the cytotoxicity assay against various types of cell lines have been provided in Figure 3. As is obvious from Figure 3, the highest cytotoxicity has been shown by the test compound against HCT-116, while the lowest cytotoxicity potential has been exhibited against MDA-MB-231. We can see from the results that at the highest concentration i.e., at 1000 ppm the results of the test compound against almost all of the cell lines are comparable with the standard drug i.e., doxorubicin. The IC_{50} values exhibited by the test compound against HCT-116, MDA-MB-231, NIH/3T3 and MCF-7 were 78, 231, 181 and 277 µg/mL respectively. The test sample has also been screened against the normal cell line i.e., WI-38 to figure out cytotoxicity and it revealed that at 1000 ppm the percent cytotoxicity was recorded as 16% and the IC_{50} value was calculated to be more than 3000 ppm of the test compound. So, it has been proved to be safe to a considerable extent for the normal cells. It has been revealed that the test compound was potentially active against all of the cell lines.

Anti-Cholinesterase Study

The results of anti-cholinesterase activity of the test compound and the positive control are summarized in

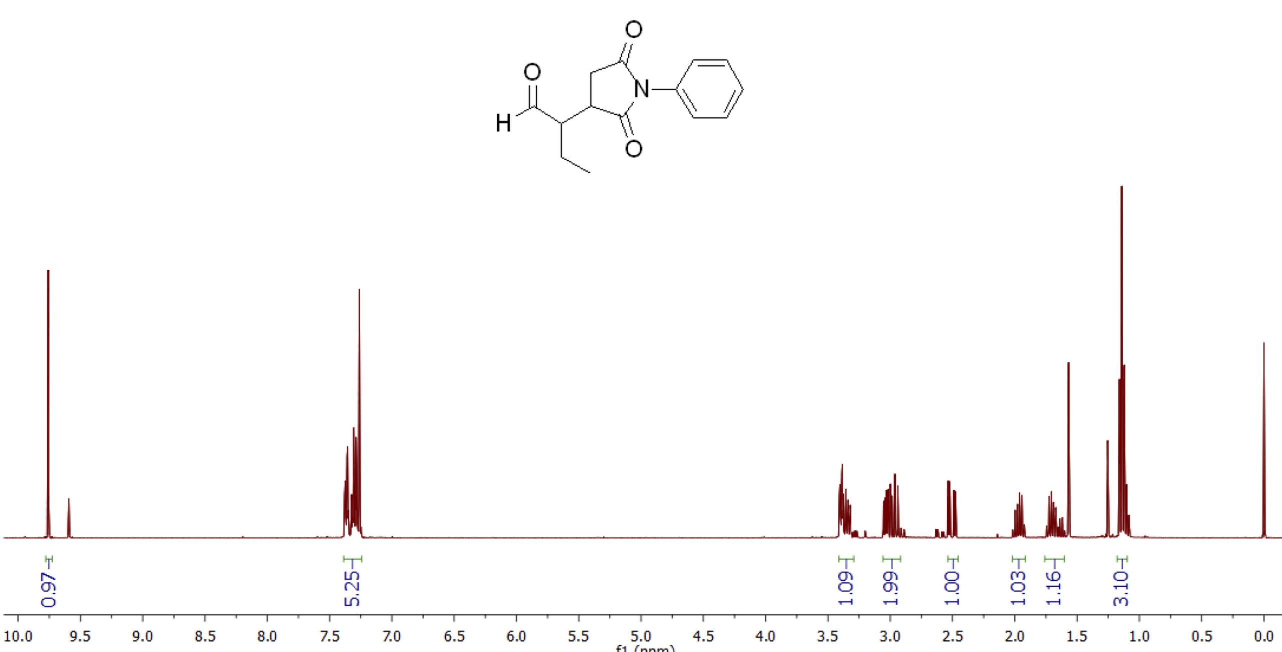

Figure 1 ^{1}H NMR spectrum of 2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)butanal.

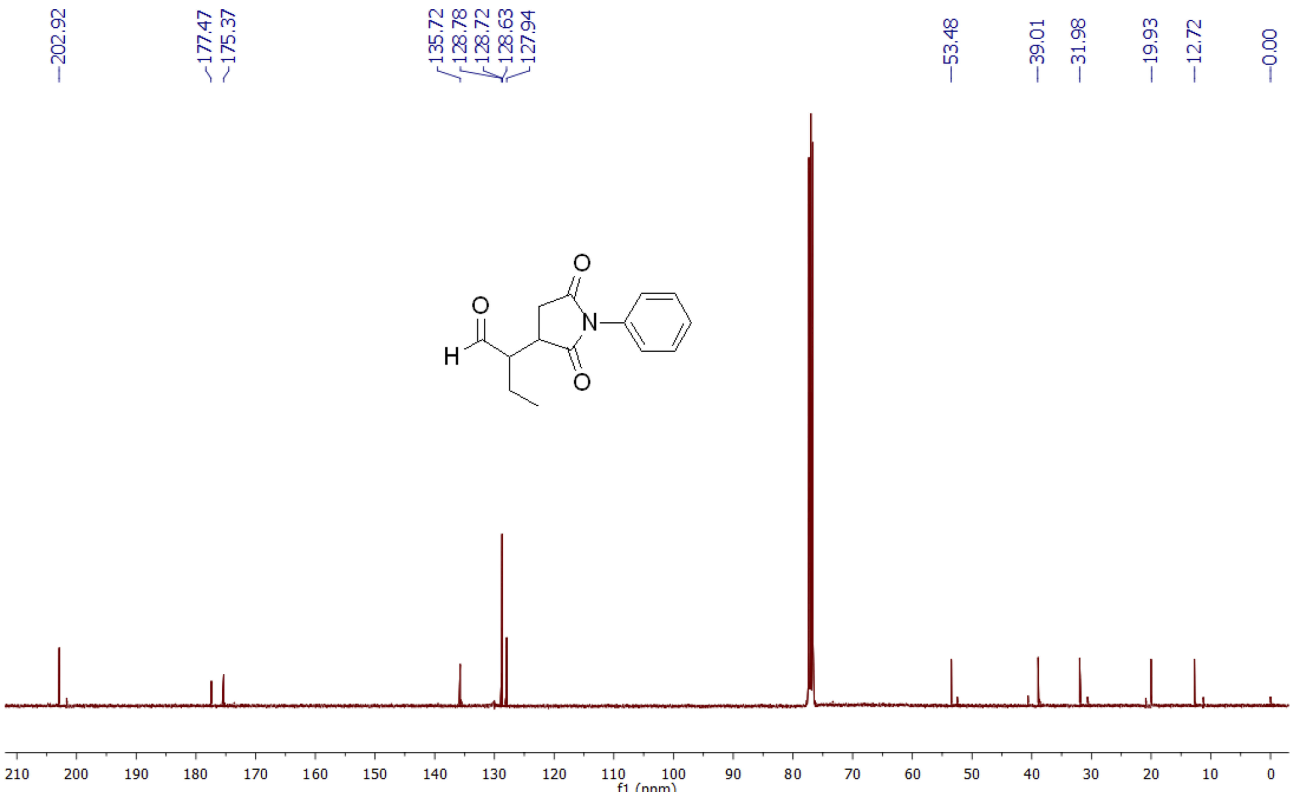

Figure 2 ^{13}C NMR spectrum of 2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)butanal.

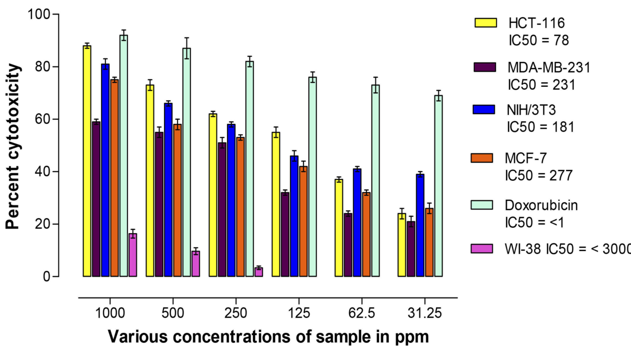

Figure 3 Percent cytotoxicity potential of test compound against various cell lines.

Table 1. 2-(2,5-Dioxo-1-phenylpyrrolidin-3-yl)butanal showed prominent anti-cholinesterase potential against both AChE and BChE. The %anti-AChE and BChE potentials exhibited by the test compound were very comparable with the standard drug. The IC_{50} values of the test compound against AChE and BChE were calculated to be 103.6 and 132.3 μg/mL, while the positive control showed the IC_{50} values of 9.8 μg/mL against AChE and 7.1 μg/mL against BChE. This shows the effectiveness of newly synthesized succinimide derivative against Alzheimer's disease.

α-Glucosidase Inhibition Study

The results of the α-glucosidase inhibitory assay have been summarized in Table 2. The percent inhibitory potential of the sample has been recorded to be nearly comparable with the standard drug i.e., acarbose. Our sample and positive control showed the percent inhibition of 79.9 and 86.7% respectively at 1 mg/mL while at the lowest concentration i.e., at the concentration of 31.25 μg/mL, the test compound and positive control exhibited 18.7 and 59.7% α-glucosidase inhibition. The IC_{50} values for the compound and positive control were observed to be 162.6 μg/mL and <1 μg/mL respectively.

Antioxidant Studies on ABTS, DPPH and H_2O_2

The antioxidant potential of 2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)butanal along with the positive control has been summarized in Table 3. The test compound showed nearly comparable free radicals scavenging potential against all three types of free radicals. Against ABTS the compound exhibited the IC_{50} value of 84.36 μg/mL, while the positive control showed 18.04 μg/mL against ABTS. Similarly, against DPPH the compound demonstrated an

Table 1 Results of Acetylcholinesterase and Butyrylcholinesterase Inhibitory Potentials of 2-(2,5-Dioxo-1-Phenylpyrrolidin-3-Yl) Butanal

Compound	Concentration (μg/mL)	Acetylcholinesterase (AChE)		Butyrylcholinesterase (BChE)	
		Percent Inhibition (Mean±SEM)	IC_{50} (μg/mL)	Percent Inhibition (Mean±SEM)	IC_{50} (μg/mL)
2-(2,5-Dioxo-1-phenylpyrrolidin-3-yl) butanal	1000	71.3±1.9***	103.6 ±4.4	73.4±1.9***	132.3 ±7.5
	500	65.4±2.2***		64.3±0.3***	
	250	61.2±1.8***		52.7±1.7***	
	125	55.9±1.1***		49.3±1.3***	
	62.5	39.6±1.7***		36.9±1.9***	
Galanthamine	1000	93.6±1.1	9.8±2.2	95.0±0.0	7.1±1.9
	500	85.4±0.2		89.4±0.5	
	250	77.9±2.3		82.8±1.5	
	125	71.9±1.5		78.6±1.7	
	62.5	68. 1±0.5		71.4±0.9	

Notes: Values significantly different in comparison with galanthamine. ***P<0.001.

Table 2 Percent α-Glycosidase Inhibitory Potential of 2-(2,5-Dioxo-1-Phenylpyrrolidin-3-Yl)Butanal

Samples	31. 25 μg/mL	62.5 μg/mL	125 μg/mL	250 μg/mL	500 μg/mL	1000 μg/mL	IC_{50} μg/mL
Test compound	18.7± 2.6***	28.7±1.9***	47.4±2.4***	58.6±3.2***	72.3±1.5**	79.9±2.5*	162.6±7.4
Acarbose	59.7±1.7	67.3±2.8	71.7±1.7	74.4± 2.3	81.7±2.6	86.7±2.6	<1

Notes: ***P<0.001, **P<0.01, *P<0.05.

Table 3 Antioxidant Activity Result of 2-(2,5-Dioxo-1-Phenylpyrrolidin-3-Yl)Butanal

S. No.	Concentration (μg/mL)	ABTS		DPPH		H_2O_2	
		% Inhibition (Mean±SEM)	IC_{50} value (μg/mL)	% Inhibition (Mean±SEM)	IC_{50} value (μg/mL)	% Inhibition (Mean±SEM)	IC_{50} value (μg/mL)
2-(2,5-Dioxo-1-phenylpyrrolidin-3-yl) butanal	1000	75.84±1.58*	84.36	72.85±1.17*	139.74	54.82±1.82***	752.21
	500	68.35±1.96***		71.36±1.92*		46.08±1.36***	
	250	61.28±0.84***		61.41±2.36***		39.92±2.08***	
	125	57.22±1.63***		49.01±1.28***		31.58±1.40***	
	62.5	48.08±2.19***		33.32±2.04***		26.08±2.66***	
Ascorbic acid	1000	81.85±0.18	18.04	83.53±0.20	11.39	78.00±1.15	29.73
	500	76.59±0.30		78.62±0.17		74.67±0.67	
	250	69.75±0.14		73.42±0.11		68.85±1.85	
	125	64.47±0.49		66.20±0.15		61.33±1.33	
	62.5	59.12±0.34		61.35±0.18		56.67±1.67	

Notes: ***P<0.001, *P<0.05.

IC_{50} value of 139.74 μg/mL and the positive control was observed with an IC_{50} value of 11.39 μg/mL. Moreover, against the third free radical, the scavenging potential was observed to be moderate in comparison to the positive control. The IC_{50} values of 752.21 μg/mL for the test compound and 29.73 for the positive control against H_2O_2 show that against the H_2O_2 free radical, the compound is moderately active.

Anthelmintic Studies

The anthelmintic activity of our test sample has shown considerable wormicidal activity. The paralysis time and death time of our sample against both test worms i.e., *P. posthuma* and *A. galli* have been recorded to be lower than those of the positive control (albendazole). Against *P. posthuma* the paralysis time and death time observed at highest concentrations i.e., 40 mg/mL were 2.48 and 14.71

Table 4 Anthelmintic Activity of 2-(2,5-Dioxo-1-Phenylpyrrolidin-3-Yl)Butanal

Samples/ Groups	Concentration (mg/mL)	*Pheretima posthuma*		*Ascaridia galli*	
		Paralysis Time (Min)	Death Time (Min)	Paralysis Time (Min)	Death Time (Min)
Compound	10	06.67±1.08	23.67±1.52	06.54±1.67	18.42±1.70
	20	05.68±0.34	19.23±2.56	04.82±1.38	11.36±1.94
	40	02.48±1.62	14.71±1.73	02.19±0.96	09.39±1.48
Alb	10	09.08±1.56	48.70±2.06	11.26±2.81	36.32±1.36
	20	07.24±1.38	39.33±1.54	09.45±1.63	32.56±1.93
	40	04.67±0.57	28.92±2.68	07.18±1.74	25.68±3.62

Note: Compound: 2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)butanal.
Abbreviation: Alb, Albendazole.

min while for the positive control the paralysis time and death time were observed to be 4.67 and 28.92 min, which shows the significance of the test compound versus the positive control. Similarly, against *A. galli*, the compound has shown extraordinary significance over the standard drug as shown in Table 4.

Discussion

The results of the current investigational studies reveal that our test sample could be one of the prominent bioactive derivatives of succinimide. AChE and BChE have been targeted to evaluate its potential role in the Alzheimer's disease (AD). Patients with AD show a very low concentration of acetylcholine (ACh) at neuronal synapses throughout the nervous system. The amount of ACh could be increased by inhibiting the enzyme responsible for the breakdown of ACh i.e., AChE. Anticholinesterase drugs are available on the market which are used for the management of AD as there is no ultimate cure for AD.[39] AD is also associated with the degeneration of neurons and the degeneration of neurons has been associated with the increased oxidative stress which in turn is associated with an increased amount of free radicals in the body. So scavenging the free radicals and decreasing the oxidative stress in the body could in turn decrease the chances of neuronal degeneration and hence decrease the chances of neurodegenerative disorders including AD.[34,40,41] In the current study we have got a significant inhibitor of free radicals and AChE as obvious from the results. In the same way, the MTT assay could be correlated with cancer therapy in such a way that the MTT assay specifies the interaction of test compounds with cancer cells. In this assay, the viable cells and cells going through cytotoxicity are sorted out. The test compound has also been proved to be safe for the normal cell line to a considerable extent as it revealed the negligible cytotoxicity against WI-38. Moreover, the compound has also been proven to significantly inhibit α-glucosidase. The α-glucosidase is actually a carbohydrase which is involved in the breakdown of carbohydrates into glucose units. So, by inhibiting this enzyme, the carbohydrates will no longer be available in the smallest units to be absorbed from the gastrointestinal tract and this will ultimately decrease the concentration of glucose in the blood. The test compound in the current investigations has revealed a very significant anthelmintic potential by killing the test worm in a very short time. The test worms employed were *P. posthuma* which is known as earthworm and *A. galli* which is the worm of fowls. *P. posthuma* and *A. galli* have close physiological and anatomical resemblance with *A. lumbricoides*. That is why these worms have been exploited in the anthelmintic screening of our sample.

Conclusions

Based on the background data and current investigations of succinimide derivative, it may be deduced that 2-(2,5-dioxo-1-phenylpyrrolidin-3-yl)butanal is a potentially bioactive compound and could be an important drug candidate against cancer, worms, diabetes mellitus and Alzheimer's disease, after going through further screening and evaluations.

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Disclosure

The authors report no conflicts of interest in this work.

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