SYNTHESIS AND IN-VITRO DETERMINATION OF NEUROPROTECTIVE EFFECT OF NOVEL 2-ANILINO-N-(2,4'-DIOXO-1,2-DIHYDROSPIRO[INDOLE-3,2'-[1,3] THIAZOLIDINE]-3'-YL) AGAINST PARKINSON'S *DISORDER* ON SH-SY5Y CELL LINES.

Sri Ramya Totakura[1], Darakhshan Begum[2], Gade Sammaiah[3]*

Abstract:
A series of novel 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiro[indole-3,2'-[1,3]thiazolidine]-3'-yl) acetamides VII-(a-j) hybrids were synthesized by conventional method via Schiff's base and cyclisation mechanism. The synthesis of novel targets with antimicrobial and antiviral activities is a core objective today in the context of the COVID-19 pandemic situations. All synthesized structures were confirmed by FT-IR, ^{1}HNMR, LC-MS, and ^{13}CNMR spectral data and the percentage yield of the title compounds was between 50-95%. The newly synthesized hybrids were assessed for their antioxidant activity by DPPH method and neuroprotective activity (the Parkinson activity) by using SH-SY5Y cell lines through MTT assay method. The damage to SH-SY5Y cell lines by 6-OHDA (6-hydroxydopamine) is an established cellular model of Parkinson's disease. From the results of neuroprotective study, all synthesized compounds showed good activity against 6-OHDA induced neurotoxicity at 31.25ug/ml concentrations and compounds **VII-a** (**75.13), VII-b** (**74.91)** and **VII-h** (**72.01)** possessed highest % cell viability. Compound **VII-c** has shown highest percentage of free radical scavenging activity with an of IC_{50} of **10.10µM**.

Keywords: Substituted Isatin, Thiazolidne, Antioxidant and Neuroprotective activities, DPPH, MTT assay, SH-SY5Y Cell line.

[1,2,3*]Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana-506009, India.

***Corresponding Author:** Gade Sammaiah
*Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana-506009, India.

DOI: - 10.48047/ecb/2023.12.si5a.0375

INTRODUCTION.

Fused heterocyclic or heterocyclic rings are one of the most important elements of the structure of many neuroprotective drugs in the market. The incorporation of thiazolidine or Indole into the molecules may affect the lipophilicity of the molecules and its ability to form hydrogen bonds.

The use of the heterocyclic ring of Indole-2-one and thiazolidine in the design of drugs can improve the pharmacological and pharmacokinetic parameters or reduce the toxicity of molecules [1-3]. Now a day's research is focusing towards the introduction of new and safe therapeutic agents of clinical importance. The heterocyclic compounds are prioritized because of their importance as being the center of activity. The Indole or Istain nucleus is one of the most important and well known heterocycles which is a common and integral feature of a variety of natural products and medicinal agents. A large number of heterocyclic compounds containing a symmetrical indole, indole-2-one and thiazolidine rings are associated with diverse pharmacological activities such as antiviral, antimicrobial, anti-Inflammatory, and anti- tumor [4].

Schiff bases are a series of compounds containing an azomethine group (-HC=N-) and showing an extensive range of biological activity.

Structurally, Schiff bases are nitrogen analogs of aldehydes and ketones, in which carbonyl moiety have been replaced by using azomethine or imino groups. Substances containing this structure have been proven to have antifungal, antibacterial, antiviral, antimalarial, cytostatic, anti-inflammatory, and antipyretic properties [5-7]. Due to this range of activities and synthesis methods that allow for the easy multidirectional modification of Schiff base structures, compounds from this group are a promising area for searches for new drugs.

The SH-SY5Y cell line is a simple and inexpensive in vitro experimental model for studying Alzheimer's disease (AD) and Parkinson disease (PD). This experimental model is a useful tool for elucidating pathophysiological mechanisms of PD and in the development of new pharmacological therapies. Parkinson's disease (PD) is a progressive, age-related, neurodegenerative disorder characterized by loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) with the formation of α-synuclein rich Lewy bodies. The exact mechanism behind the pathogenesis and SNpc cell death in PD is still unclear but at the molecular level, oxidative stress, mitochondrial dysfunction, ubiquitin-proteasome system impairment, dopamine metabolism and neuroinflammation are thought to be involved [8]. Mitochondrial dysfunction has been associated with ageing and age-related disorders including Parkinson's disease [9-10]. Post-translational modification of mitochondrial proteins is one of the mechanisms that play a role in modulation of mitochondrial activity.

The most common immortalized cell line used in this type of research is the human-derived SH-SY5Y cell line. The SH-SY5Y is a subline of cells isolated from the bone marrow from a metastatic neuroblastoma of a 4-year-old female [11]. The cell line has a catecholaminergic phenotype, equipped with tyrosine hydroxylase and dopamine beta-hydroxylase enzymes, and is able to synthesize both dopamine and noradrenaline neurotransmitters [12]. In addition, SH-SY5Y can be further differentiated into a more mature dopaminergic phenotype [13]. Thus, these characteristics make this cell line a suitable in vitro model for the study of Parkinson Disease (PD).

Based on the above literature data, we decided to design and synthesize a series of compounds that are new derivatives of 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiri[indole-3,2'-[1,3] thiazolidine]-3'-yl) acetamides VII-(a-j) hybrids with the character of Schiff bases derived from different Isatins. The aim of developing this new series of compounds was to find new antioxidant and Parkinson drugs that are effective against SH-SY5Y Cell lines.

EXPERIMENTAL SECTION.

Materials and Methods

In this research chemicals were purchased from local dealer with SD Fine chem, Himedia and Sigma-Aldrich. All chemicals were 97-99% pure; purity of the synthesized compounds has been checked by TLC and melting point was carried out by using Thieles tube apparatus. The conventional methods have been used for synthesis of novel 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiri- [indole-3,2'-thiazolidine]-3'-yl)acetamides VII -(a-j) hybrids respectively.

The IUPAC nomenclature of synthetic compounds VII-(a-j) was given by using Chem draw Ultra-12 version. The final title compounds VII-(a-j) were synthesized in six steps using different reagents, solvents, and reaction conditions. The structure was established by spectral data (IR, ^{1}HNMR, ^{13}CNMR and Mass.

General Procedure:

Step-I: Synthesis of Isonitrosoacetanilides (II): Isonitrosoacetanilides are obtained from different substituted anilines. 5g/5ml of anilines were taken into a conical flask along with 30ml of water. To

this 5ml of conc. hydrochloric acid was added. In another beaker 9ml of chloral hydrate was added. Further 12g of hydroxylamine hydrochloride was added along with 30ml of water. The mixture was stirred well and anhydrous sodium sulphate was added until a precipitate appeared. The contents of the beaker were then mixed with those in conical flask and heated on a water bath for about 45min and left aside for 24hours. After 24 hours, the mixture was filtered and washed with water until acid free and dried completely.

Step-II: Synthesis of substituted Isatin derivatives (III-(a-j): The isonitrosoacetanilides thus obtained in Step-I were further cyclized with Conc.H_2SO_4 which was heated earlier to about 80°C by adding the isonitrosoacetanilide in smaller portions until it dissolves completely and the mixture was left overnight. After 24 hours, crushed ice was added to the mixture and the product thus obtained was filtered and washed with water until the red litmus turned blue.
All isatins were prepared similarly and dried completely. Purification of the compound was effected by the recrystallization from methanol. Various derivatives of indole-2,3-diones were prepared by using different aromatic amines and were confirmed by TLC.

Step-III: Synthesis of Ethyl anilinoacetate (IV): Equimolar quantities of Aniline and Ethyl chloroacetate were taken into a round bottomed flask. To the reaction mixture, dry acetone (25 ml) in presence of anhydrous potassium carbonate (0.01 mol) was added and refluxed for 10hours on water bath with the help of double condenser. The progress of the reaction was checked through TLC. After concluding the completion of reaction, the reaction mixture was poured into a cleaned petriplate and left for evaporation. The resultant gummy product was washed with petroleum ether and the compound was dried and collected. It was further purified by recrystallization from petroleum ether to get a brown crystalline solid, m.p.55°C.

Step-4: Synthesis of 2-anilinoaceto hydrazide (V): A mixture of ethyl anilinoacetate (IV, 0.01mol) and hydrazine hydrate (0.05 mol) were taken into a RB flask using methanol as the solvent (25 ml). The mixture was heated in reflux condenser for 6-8 hours. The reaction mixture was cooled and solvent was removed by evaporation. The concentrated reaction mixture was poured on to crushed ice to get the product. The molecule was separated by filtration, washed with ice cold deionized water and dried. The compound was recrystallized from methanol to get a colorless crystalline solid with yield of 95%, m.p. 125-127°C.

Step-5: Synthesis of 2-*N*'-[2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] acetohydrazide (VI): Equimolar concentration of 2-anilino aceto hydrazide (V, 0.01 mol) and different isatins (III 0.01 mol) were heated under reflux in methanol for 12 hours. After confirming the end of reaction by TLC, the resultant reaction mixture was poured onto crushed ice. The formed solid was filtered and dried and purified by recrystallization from ethanol. Fifteen (15) different intermediates were prepared by this procedure.

Step-6: Synthesis of 2-anilino-*N*-(2,4'-dioxo-1,2-dihydrospiro[indole-3,2'-[1,3] thiazolidin]-3'-yl)acetamides [VII-(a-j)]: To the above intermediate formed, thioglycolic acid (0.002 mol) was added along with 10 ml of glacial acetic acid and finally a pinch of Zinc chloride in the presence of Methanol as solvent in a round bottomed flask. The mixture was refluxed for about 12-24hours and poured onto crushed ice. The solid formed was separated through filtration and washed with 10%Na_2CO_3 solution to remove any remainants of thioglycolic acid. The filtrate was further washed with cool water and dried. The dried product was purified by recrystallization from methanol/ ethanol. All the remaining derivatives were synthesized in the similar way.

Compound. VII-a: N-(2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)-2-(phenylaminoacetamide. IR (KBr) cm^{-1}: 3386.21(N-H str), 3098.32(-C-H *Str*, Aromatic), 2989.21 (-CH *Str* in Aliphatic), 1710.21(-CO *Str* in Ketone group), 1479.23(-C=C *Str* in Aromatic), 1252.14(-CS Str in Thiazolidine ring). ^{1}H-NMR($CDCl_3$-D) δ ppm: 4.094-4.082(s, 2H, -CH_2-CO), 4.673(s, 2H, -CH_2 Thiazolidine proton), 7.184-7.108(t, 2H, Ar-H), 7.262-7.211(d, 2H, Ar-H), 7.678-7.380(t, 2H, Ar-H), 7.810-7.759 (d, 2H, A-H), 11.982(s, 1H, -NH-), 12.575(s, 1H, -NHCO-), 13.129(s, 1H, -NH in Indole). ^{13}CNMR (CDCl3-D): 172.03, 168.34, 167.21, 148.41, 137. 34, 134.29, 128.03, 126.23, 124.13, 123.32, 121. 45, 118.34, 116.03, 70.54, 47.92, 36.13. Mass (EI-MS): m/z 368.01(M), 369.03(M + 1, 100%).

Compound.VII-b: Spectral data of N-(5-methyl-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl)-2-(phenyl amino) acetamide (VII-b):IR (KBr) cm^{-1}: 3440.99(N-H str), 3068 ,56(Ar C-H str), 2827.71(Aliphatic C-H str), 1742. 90(C=O),

744.90(C-Cl). ^{1}H-NMR($CDCl_3$-D) δ ppm: 2.02(s, 3H, Ar-CH_3); 3.52 (s, 2H, CH_2, Thiazolidine), 3.62(s, 2H, CH_2), 3.83(s, 2H, -CH_2-CO), 6.09(t, 1H, NH), 6.55(d, 2H, Ar-H), 6.57(t, 1H, Ar-H), 6.71(t, 2H, Ar-H), 7.06(d, 1H, Ar-H), 7.15(d, 1H, Ar-H), 7.17(s, 1H, Ar-H), 8.54(s, 1H, CONH), 8.66(s, 1H, NH proton in indole). ^{13}CNMR (CDCl3-D): 176.18, 171.95, 167.95, 147.08, 140.80, 129,96, 129.51, 129.91, 125.93, 124.97, 11740, 113.49, 112.85, 70.94, 46.48, 34.04. Mass (EI-MS): m/z 382.04(M, 100%), 384.05(M + 2, 30%).

Compound. VII-c: Spectral data of N-(7-methyl-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl)-2-(phenyl amino) acetamide (VII-c): IR (KBr) cm^{-1}: 3402.32(N-H str), 3076.34(-C-H *Str*, Aromatic), 2998.32(-CH *Str* in Aliphatic), 1714.02(-CO *Str* in Ketone group), 1486.32(-C=C *Str* in Aromatic), 1248.21(-CS Str in Thiazolidine ring). ^{1}H-NMR($CDCl_3$-D) δ ppm: 2.273(s, 3H, Ar-CH_3), 4.083(s, 2H, -CH_2-CO), 4.455-4.410(s, 2H, -CH_2 Thiazolidine proton), 7.189-7.144(t, 2H, Ar-H), 7.262-7.207(t, 2H, Ar-H), 7.538-7.520(d, 2H, Ar-H), 7.897-7.654(d, 2H, A-H), 11.876(s, 1H, -NH-), 12.569(s, 1H, -NHCO-), 13.659(s, 1H, -NH in Indole). ^{13}CNMR(CDCl3-D): 178.32, 164.21, 164.29, 145.01, 135.23, 130.54, 129.93, 128.32, 125.02, 124.12, 120.12, 119.21, 115.65, 70.76, 48.43, 38.39. Mass (EI-MS): m/z 382.04(M), 383.12(M + 1, 100%).

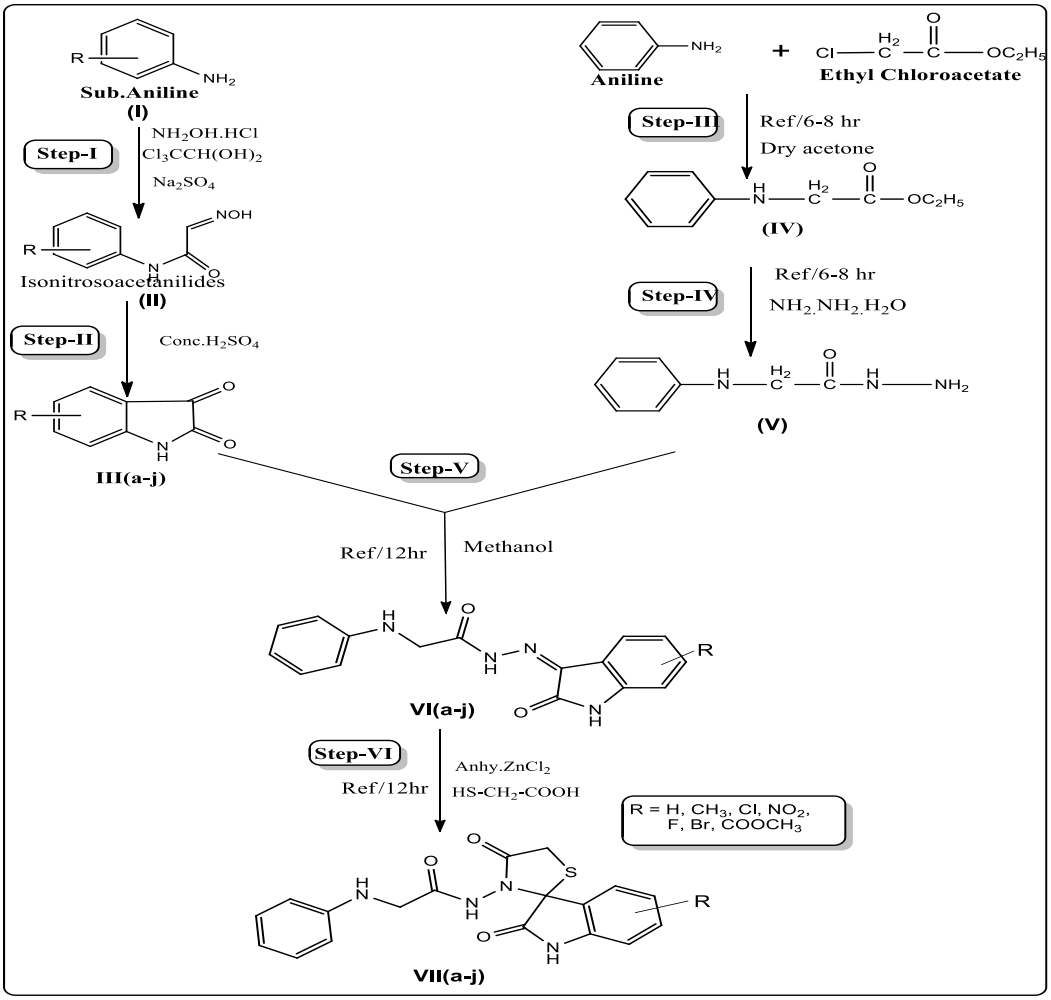

Figure 1. Scheme for the synthesis novel 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiri[indole-3,2'-[1,3]thiazolidine]-3'-yl) acetamides VII-(a-j)

Compound.VII-d: Spectral data of N-(5-chloro-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl)-2-(phenyl amino) acetamide (VII-d): IR (KBr) cm^{-1}: 3440.99(-NH *Str*), 3068.56(-CH *Str*, Aliphatic), 2827.71(-C-H *Str*, Aliphatic), 1742.90 (-CO Str, Ketone); 1398.20(-C=C Str),1222(-CS Str), 744.90(-C-Cl *Str*, Ar-Cl). ^{1}H-NMR($CDCl_3$-D) δ ppm: 3.52(s, 2H, -CH_2-CO protons), 3.62(s, 2H, -CH_2 protons Thiazolidine), 3.83(s, 2H, -CH_2), 6.09(t, 1H, -NH), 6.55(d, 2H, Ar-H), 6.57(t, ^{1}H, Ar-H), 6.71(t, 2H, Ar-H), 7.06(d, 1H, Ar-H), 7.15(d, 1H, Ar-H), 7.17(s, 1H, Ar-H), 8.54(s, 1H, -CONH), 8.66(s, 1H, -NH protons in indole).

^{13}CNMR($CDCl_3$-D): 176.18, 171.95, 167.95, 147.08, 140.80, 129,96, 129.51, 129.91, 125.93, 124.97, 117.40, 113.49, 112.85, 70.94, 46.48, 34.04. Mass (EI-MS): m/z 402.32(M), 403.54(M+1, 100%), 404.04(M+2, 30%).

Compound.VII-e: Spectral data of N-(7-chloro-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl)-2-(phenyl amino) acetamide (VII-e): IR (KBr) cm^{-1}: 3387.32(-NH *Str*), 3076.18(-CH *Str*, Aromatic), 2889.54(-C-H *Str*, Aliphatic), 1705. 18(-CO *Str*, Ketone), 1532(-C=C *Str*, Aromatic), 812(-C-Cl *Str*, Ar-Cl). ^{1}H-NMR ($CDCl_3$-D) δ ppm: 3.654(s, 2H, -CH_2-CO protons), 3.764(s, 2H, -CH_2 protons Thiazolidine), 3.987(s, 2H, -CH_2), 6.13(t, 1H, -NH), 6.7643(d, 2H, Ar-H), 6.983(t, 1H, Ar-H), 7.843(t, 2H, Ar-H), 7.983(d, 1H, Ar-H), 8.002(d, 1H, Ar-H), 8.093(s, 1H, Ar-H), 9.093(s, 1H, -CONH), 10.32(s, 1H, -NH protons in indole). ^{13}CNMR($CDCl_3$-D): 174.09, 170.21, 168.43, 147.012, 144.63, 134,21, 131.65, 128.32, 124.03, 128.90, 124.03, 120.12, 117.05, 73.32 48.32, 36.02. Mass (EI-MS): 402.32(M), 403.54(M+1, 100%), 404.04(M+2, 30%).

Compound.VII-f: Spectral data of N-(5-fluoro-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl) -2-(phenyl amino) acetamide (VII-f): IR (KBr) cm^{-1}: 3434.55(-NH *Str*), 3098.33(-CH *Str*, Aromatic), 2988.89, 2915.35(-C-H *Str*, Aliphatic), 1729.14(-CO *Str*, Ketone), 1434.98(-C=C *Str*, Aromatic), 1247.11(-CS *Str*), 1217.26(-C-F *Str*, Ar-F). ^{1}H-NMR($CDCl_3$-D) δ ppm: 3.453(d, 1H, -CH_2 protons in Thiazolidine), 3.764(d, 2H, -CH_2 protons Thiazolidine), 4.446(s, 2H, -CH_2), 6.598-6.529(t, 3H, Ar-H), 6.885-6.853 (d, 2H, Ar-H), 7.695-7.625(d, 2H, Ar-H), 7.715(s, 1H, Ar-H), 9.057(s, 1H, -CONH), 10.675(s,1H, -NHCO), 13.213(s, 1H, -NH protons in indole). ^{13}CNMR($CDCl_3$-D): 178.03, 174.43, 169.32, 147.43, 142.98, 137.83, 135.23, 132.94, 128.54, 126.12, 125.43, 123.32, 120.33, 78.21 46.76, 38. 43. Mass (EI-MS): m/z 386.02(M), 387.21(M+1, 100%).

Compound.VII-g: Spectral data of N-(5-Bromo-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl)-2-(phenyl amino) acetamide (VII-g): IR (KBr) cm^{-1}: 3431.72(N-H str), 3102,16(Ar C-H str), 2923.98, 2853.24(Aliphatic C-H str), 1649.51(C=O), 1383.75(-C=C *Str*); 1252.51(-CS *Str*); 1032(-C-Br Str). ^{1}H-NMR ($CDCl_3$-D) δ ppm: 3.68(d, 1H, -CH_2 Thiazolidine), 3.72(d, 1H, -CH_2 Thiazolidine), 4.03(s, 2H, -CH2), 6.27(t, 1H, NH), 6.75(d, 2H, Ar-H), 6.88(t, 1H, Ar-H), 7.24(t, 2H, A-Hr) 7.50(t, 1H, Ar-H), 7.83(d, 1H, Ar-H), 8.08(d, 1H, Ar-H), 8.95(S, 1H, CONH), 9.02(S, 1H, NH proton in indole). ^{13}CNMR(CDCl3-D): 172.19, 168.72, 167.95, 147.08, 138.69, 133.92, 130.16, 129.51, 127.34, 126.11, 121.76, 117.40, 113.49, 70.94, 46.48, 34.04. Mass (EI-MS): m/z 447.14(M), 449(M + 1, 100%).

Compound.VII-h: Spectral data of N-(5-nitro-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl)-2-(phenyl amino) acetamide (VII-h):): IR (KBr) cm^{-1}: 3394.37(N-H str),3102,16(Ar C-H str),2916.06, 2824.50(Aliphatic C-H str),1666.79(C=O); 1510.16(-N-O Str); 1256.12(-CS *Str*). ^{1}H-NMR($CDCl_3$-D) δ ppm: 3.68(d, 1H, -CH_2, Thiazolidine), 3.72(d, 1H, -CH_2, Thiazolidine), 4.03(s, 2H, -CH_2), 6.27(t, 1H, NH), 6.75(d, 2H, Ar-H), 6.88(t, 1H, Ar-H), 7.24(t, 2H, Ar-H) 7.50(t, 1H, Ar-H), 7.83(d, 1H, Ar-H), 8.08(d, 1H, Ar-H), 8.95(S, 1H, CONH), 9.44(S,1H, NH proton in indole). ^{13}CNMR(CDCl3-D): 172.19, 168.72, 167.95, 147.08, 138.69, 133.92, 130.16, 129.51, 127.34, 126.11, 121.76, 117.40, 113.49, 70.94, 46.48, 34.04. Mass (EI-MS): m/z 413.23(M), 415(M + 1, 100%).

Table 1. Physical data of novel 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiri[indole-3,2'-[1,3]thiazolidine]-3'-yl) acetamides VII-(a-j)

Compound	Molecular Formula	R	Molecular Weight(gm)	Melting Point(ºC)	% Yield
VII-a	$C_{18}H_{16}N_4O_3S$	-H	368.09	198-200	78
VII-b	$C_{19}H_{18}N_4O_3S$	5-CH_3	382.11	228-230	95
VII-c	$C_{19}H_{18}N_4O_3S$	7-CH_3	382.11	222-224	80
VII-d	$C_{18}H_{15}ClN_4O_3S$	5-Cl	402.06	218-220	84
VII-e	$C_{18}H_{15}ClN_4O_3S$	7-Cl	402.06	200-202	90
VII-f	$C_{18}H_{15}FN_4O_3S$	5-F	386.08	230-232	85
VII-g	$C_{18}H_{15}BrN_4O_3S$	5-Br	446.00	258-260	95
VII-h	$C_{18}H_{15}N_5O_5S$	5-NO_2	413.08	240-242	70
VII-i	$C_{20}H_{18}N_4O_5S$	7-$COOCH_3$	426.10	240-242	75
VII-j	$C_{18}H_{14}ClFN_4O_3S$	5-F, 6-Cl	420.05	215-218	70

Compound.VII-i: Spectral data of N-(7-aceto-2,4'-dioxospiro[indoline-3,2'-thiazolidin] -3'-yl)-2-(phenyl amino) acetamide(VII-i): 3346.49(N-H str), 3054.21(-C-H *Str*, Aromatic), 2923.06,2853.00(-CH *Str* in Aliphatic), 1652.36(-CO *Str* in $COOCH_3$), 1427.94(-C=C *Str* in Aromatic), 1260.39(-CS Str in Thiazolidine ring). ^{1}H-NMR($CDCl_3$-D) δ ppm: 3.719-3.599(s, 3H, -$COOCH_3$), 4.326-4.273(s, 2H, -CH_2-CO), 4.349-4.334(s, 2H, -CH_2 Thiazolidine proton), 7.923-7.681(t, 3H, Ar-H), 7.955(t, 1H, Ar-H), 7.984-7.980(d, 2H, Ar-H), 8.005-8.001(d, 2H, A-H), 8.502(s, 1H, -NH-), 9.070(s, 1H, -NHCO-), 10.21(s, 1H, -NH in Indole). ^{13}CNMR(CDCl3-D): 178.03, 171.21, 165.43, 150.84, 136.56, 133.20, 130.32, 128.17, 126.65, 125.98, 124.45, 120.23, 119.12, 73.26, 48.21, 39.43. Mass (EI-MS): m/z 426.19(M), 427.08(M + 1, 100%).

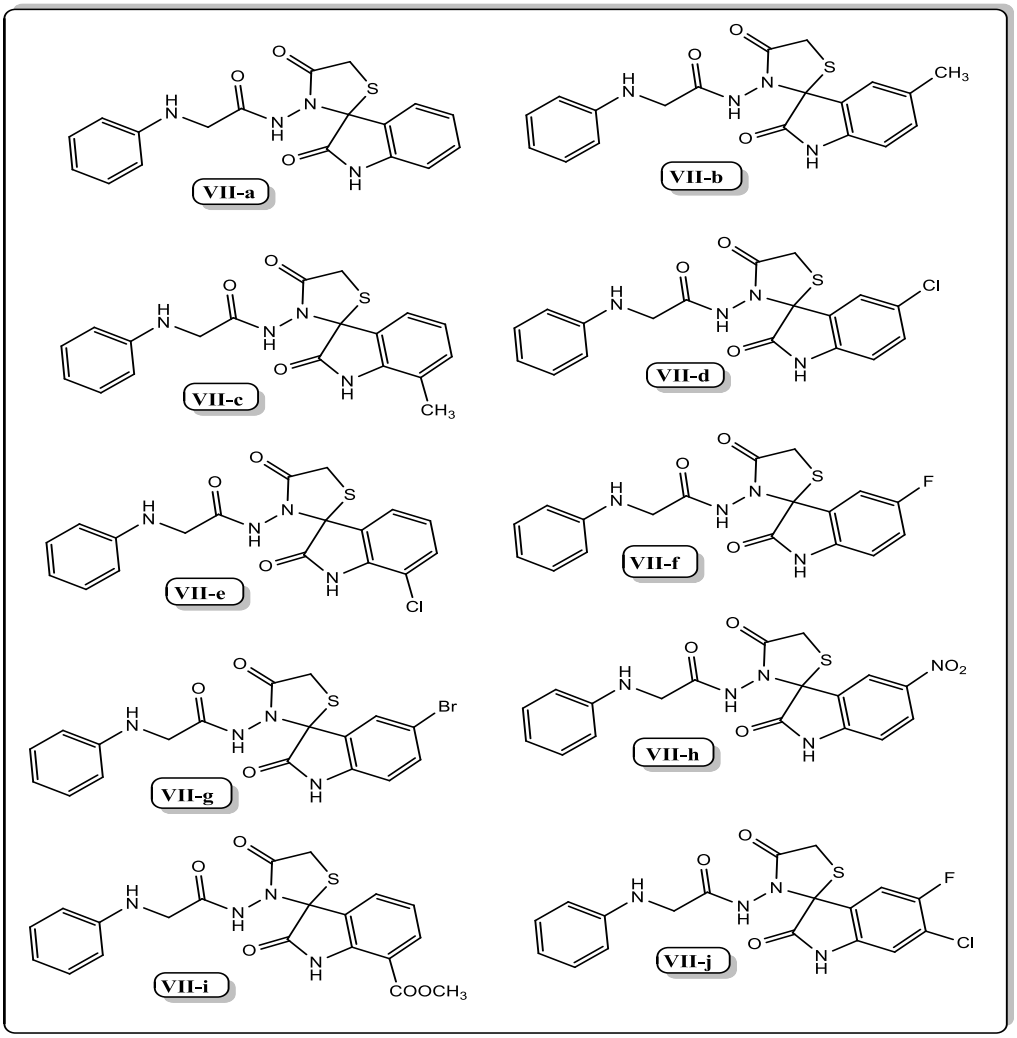

Figure 3. Novel synthesized compounds VII-(a-j)

Compound. VII-j: Spectral data of N-(5-fluor-6-chloro-2,4'-dioxospiro[indoline-3,2'-thiazoli din] -3'-yl)-2-(phenyl amino) acetamide(VII-j): 3357.92(N-H str), 3019.96(-C-H *Str*, Aromatic), 2854.04(-CH *Str* in Aliphatic), 1703.54(-CO *Str* in Ketone), 1467.03(-C=C *Str* in Aromatic), 1287. 12(-CS Str in Thiazolidine ring), 1213.82(-C-F *Str*); 749.93(-C-Cl *Str*). ^{1}H-NMR($CDCl_3$-D) δ ppm: 4.33(s, 2H, -CH_2-CO), 5.212(s, 2H, -CH_2 Thiazolidine proton), 6.993-6.903(t, 3H, Ar-H), 7.088(d, 2H, Ar-H), 7.323(s, 1H, Ar-H), 7.445 (s, 1H, A-H), 10.930(s, 1H, -NH-), 11.319(s, 1H, -NHCO-), 13.489(s, 1H, -NH in Indole). ^{13}CNMR ($CDCl_3$-D): 179.04, 170.46, 168.21, 156.87, 140.32, 138.43, 137.33, 134.28, 132.76, 128.43, 126.76, 123.23, 120.98, 76.43, 49.21, 40.36. Mass (EI-MS): m/z 420.05(M), 421.21(M + 1, 100%), 422.03(M + 2, 30%).

Pharmacological Activity:

Antioxidant activity: The synthesized 2-anilino-*N*-(2,4'-dioxo-1,2-dihydrospiro [indole-3, 2'-[1,3] thiazolidin]-3'-yl)acetamide derivatives (VII a-VII j) were evaluated for antioxidant activity by employing Ascorbic acid as the standard(reference antioxidant molecule) using the DPPH (2,2-

diphenyl -1-picryl-hydrazyl-hydrate) radical scavenging assay. DPPH in ethanol shows a strong absorption band at 517 nm (independent of pH from 5.0 to 6.5), and the solution appears to be deep violet in color. As the DPPH radical is scavenged by the donated hydrogen from the antioxidant, the absorbance is diminished according to the stoichiometry. 0.5 mL of DPPH solution (0.2 mM) was mixed with 0.1 mL of various concentrations (10µM, 20µM, 40µM, 60µM, 80µM, 100µM) of test compounds and 1.5 mL ethanol was added. The mixture was kept at room temperature for 30 min under dark condition, and then the absorbance (OD) was read at 517 nm against blank. The % reduction of free radical concentration (OD) with different concentration of test compounds was calculated and was compared with standard, ascorbic acid[14].

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of Blank - Absorbance of Test X 100}}{\text{Absorbance of Blank}}$$

The results were expressed as IC_{50} values (the concentration of test required to scavenge 50% free radicals).

Neuroprotective effect (Parkinson's disease):

SH-SY5Y Cell Culture: MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm [15].

Cell Viability Assay: Seed 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allow the cells to grow for about 12 hours. Add appropriate concentrations of the test agent (Mentioned in the results - Excel sheet). Incubate the plate for 24hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, takeout the plates from incubator, and remove spent media and add 80µM of neurotoxic agent, 6-OHDA and incubate for 3-4 hrs. After the incubation period, takeout the plates from incubator, and remove spent media and add MTT reagent to a final concentration of 0.5mg/mL of total volume.

Wrap the plate with aluminium foil to avoid exposure to light. Return the plates to the incubator and incubate for 3 hours. (Note: Incubation time varies for different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.)

Remove the MTT reagent and then add 100µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. Read the absorbance on a spectrophotometer or an ELISA reader at 570nm wavelength

RESULTS AND DISCUSSIONS.

Synthesis: The series of novel 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiri[indole-3,2'-[1,3] thiazolidine]-3'-yl) acetamides VII-(a-j) hybrids were synthesized by conventional method via Schiff's base and Cyclisation mechanism. All compounds show satisfactory analysis for the proposed structures, and which were confirmed on the basis of their FT-IR, LC-MASS, ^{1}H NMR, and ^{13}NMR spectral data. In the present work the effort is made to develop a convenient method for the synthesis of novel 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiro[indole-3,2'-[1,3] thiazolidine]-3'-yl) acetamides (Scheme-I, VII(a-j).

Spectral data: Spectral characterization of 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiro[indole-3,2'-[1,3] thiazolidine]-3'-yl) acetamides (Scheme-I, VII(a-j) derivatives was performed by IR spectroscopy. Practically, in all the compounds are showing the aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3002-3097 cm^{-1} and 2900-2743 cm^{-1}. All the compounds have been show strong absorption in the region of 1696-1723cm^{-1} is found to be presence of C=O stretching frequency and in most of the compounds the C=C stretching of the aromatic ring is around 1545-1535cm^{-1} respectively. The Ar-Cl stretching is showing the strong absorption in the region 793-827 cm^{-1} and few compounds containing -NO_2 group shows

peaks due to stretching of $–NO_2$ group is observed at around 1622-1654cm^{-1} respectively. Similarly, the ^{1}HNMR (DMSO-d6) spectra of -anilino-N-(2,4'-dioxo-1,2-dihydrospiro[indole-3,2'-[1,3] thiazolidine]-3'-yl) acetamides derivatives are showed a singlet at 10.01-12.021 for -NH in indole proton and singlet at 8.532-10.92 for -NH protons in acetamide. Some of the compounds are showing triplet at 1.943-2.3523 for $–CH_3$ in Ar-CH_3 protons. All these compounds have aromatic protons were found between δ 8.356-6.793 ppm as singlet, doublet and triplet protons.

Antioxidant activity: Thee antioxidant activity of all the synthesized compounds performed using DPPH method and the results given in Table 2. The values are expressed in IC_{50} that is, ability of the test compound required to decrease the concentration of test free radical by 50%. All the synthetic compounds produced a concentrations dependent scavenging of free radical. The IC_{50} values of all the synthetic test compounds were found ranges between **10.10 to 16.61 μM**. Among all the test compounds, compounds **VII-a, VII-b, VII-c, VII-g**, and **VII-j** had more potent antioxidant activity against DPPH free radicals. It is proposed that DPPH may be scavenged by an antioxidant through donation of hydrogen (H·) to form a stable DPPH-H molecule which does not absorb at 517 nm. Thus the results show that synthesized compound **VII-c (IC_{50}, 10.10 μM)** shown highest percentage of free radical scavenging activity. It was observed that the test compounds with electron donating groups (**7-CH_3**) on the Indole ring favors anti-oxidant activity.

Table 2. Antioxidant activity of Novel synthesized compounds VII-(a-j)- IC50 Values.

S. No	Compounds	R	IC50 Values µM
1	VII-a	H	10.45
2	VII-b	5-CH_3	10.52
3	**VII-c**	**7- CH3**	**10.10**
4	VII-d	5-Cl	15.07
5	VII-e	7-Cl	14.91
6	VII-f	5-F	13.39
7	VII-g	5-Br	10.61
8	VII-h	5-NO_2	15.6
9	VII-i	7-$COOCH_3$	16.61
10	VII-j	5-F, 6-Cl	10.98
11	Ascorbic acid	-	5.87

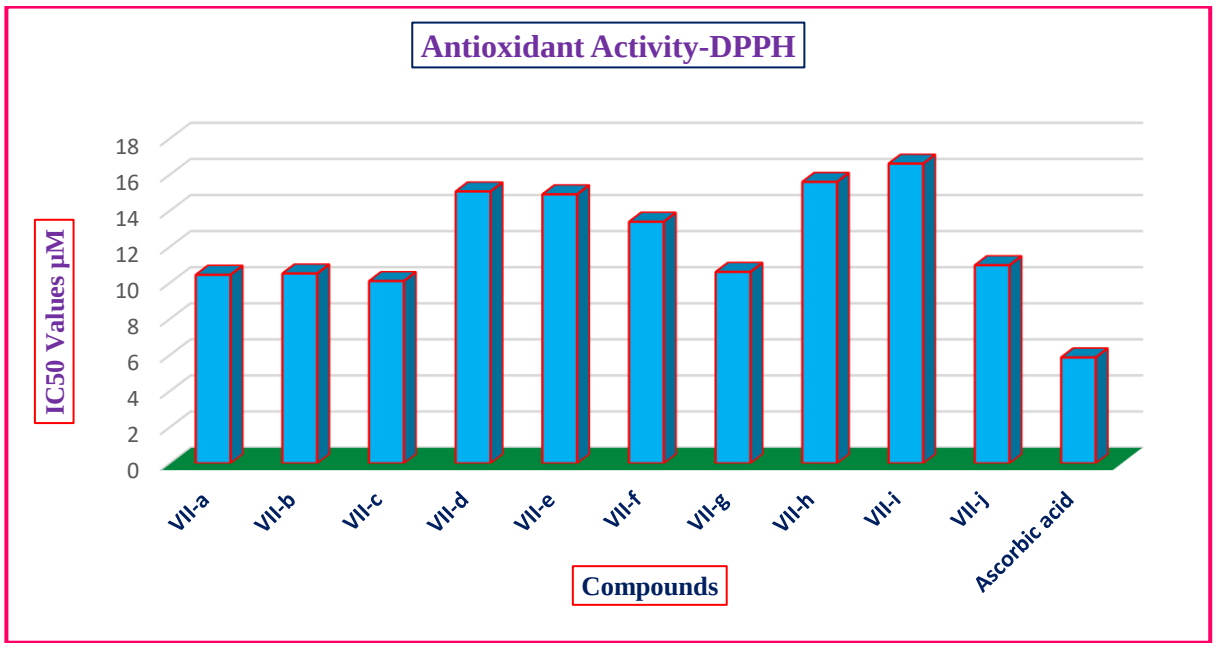

Figure 4. Graphical representation Antioxidant activity of Novel synthesized compounds VII-(a-j)- IC50 Values.

Neuroprotective effect (Parkinson's disease): The newly synthesized hybrids were assessed for their neuroprotective activity (The Parkinson activity was carried out by using SH-SY5Y cell lines) by MTT assay method. The damage to SH-SY5Y cell lines by 6-OHDA(6-hydroxydo pamine) is an established cellular model of Parkinson's disease. From the results of

neuroprotective study, all synthesized compounds showed good activity against 6-OHDA induced neurotoxicity at 31.25ug/ml concentrations and compounds **VII-a (75.13), VII-b (74.91)** and **VII-h (72.01)** possesses highest % cell viability.

Table 2. Neuroprotective activity of Novel synthesized compounds VII-(a-j) on SHSY-5 cell lines against 6-OHDA toxin for Parkinson's disease: %Cell Viability

S.NO	Compound	R	% Cell Viability			
			7.8µg/ml	15.62µg/ml	31.25µg/ml	128µg/ml
1	VII-a	**H**	64.58	66.84	**75.13**	
2	VII-b	**5-CH_3**	64.04	68.03	**74.91**	
3	VII-c	7- CH3	59.2	61.57	65.98	
4	VII-d	5-Cl	58.44	62.64	69.75	
5	VII-e	7-Cl	55.65	62.54	63.93	
6	VII-f	5-F	63.72	66.3	70.93	
7	VII-g	5-Br	58.77	61.89	63.29	
8	VII-h	**5-NO_2**	56.62	61.03	**72.01**	
9	VII-i	7-COOCH3	51.99	61.35	62.1	
10	VII-j	5-F, 6-Cl	57.15	59.74	68.89	
11	**Standard**	**LDopa**				**90.74**

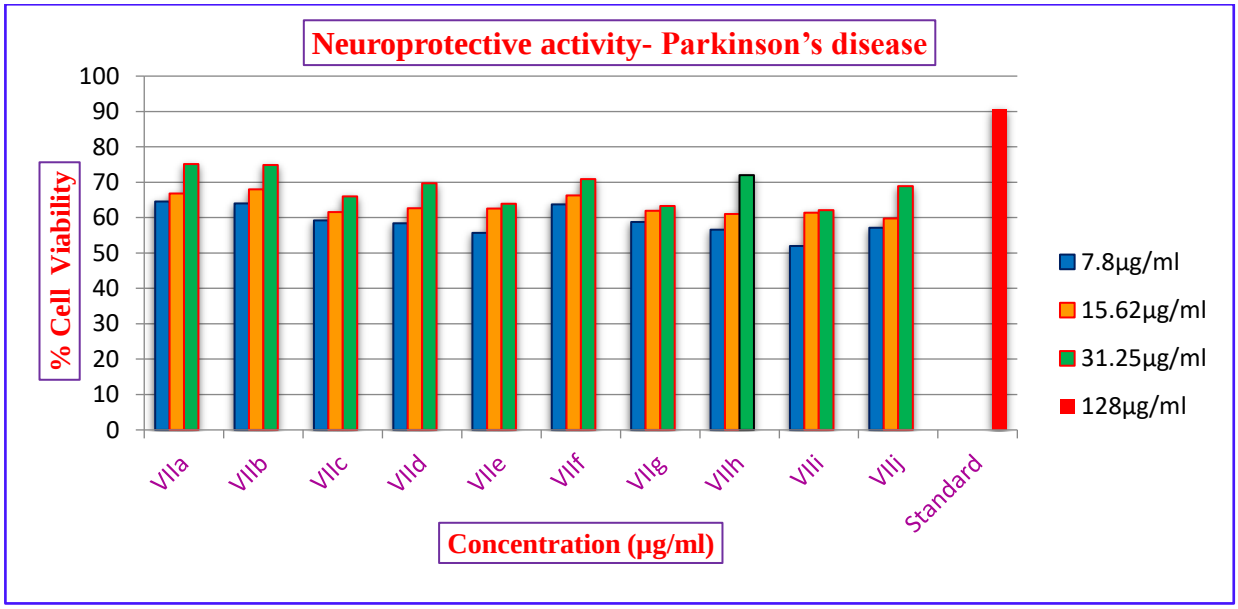

Figure 3. Graphical representation Neuroprotective activity of Novel synthesized compounds VII-(a-j) on SHSY-5 cell lines against 6-OHDA toxin for Parkinson's disease

CONCLUSION.

The 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiri[Indole-3,2'-[1,3]thiazolidine]-3'-yl) acetamides (Scheme-I, VII(a-j) derivatives were developed by a six step process. The yield of the synthesized compound was found to be in the range from 50-95**%.** All these synthesized compound structures was confirmed by IR, ^{1}H-NMR, ^{13}NMR and , Mass spectroscopy. These compounds were assessed for their antioxidant activity by DPPH method and neuroprotective activity (The Parkinson's activity was carried out by using SH-SY5Y cell lines) by MTT assay method. In conclusion, the present study highlights the importance of the derivatives of 2-anilino-N-(2,4'-dioxo-1,2-dihydrospiri [indole-3,2'-[1,3] thiazolidine]-3'-yl) acetamides (Scheme-I, VII(a-j) containing various substitutions are features responsible for the various biological activities and may serve as a lead molecule for further modification to obtain clinically useful novel entities**.**

ACKNOWLEDGEMENT: We are Thankful to all the personnel at Stellixir Biotech Pvt.Ltd, Bengaluru, a preclinical contract research organization for helping us in determining neuroprotective activity.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

REFERENSES

1. O.P. Chzhu, D.E. Araviashvili, I.G. Danilova, Studying properties of prospective biologically active extracts from Marine Hydrobionts, Emerg. Sci. J 2020; 4 (1): 37–43.
2. A. Tasneem, G.P. Rai, S. Reyaz, H.R. Bairagya, The possible molecular mechanism of SARS-CoV-2 Main Protease: new structural insights from computational methods, Sci. Med. J. 2020; 2; 108–126 (Sp. Iss. "COVID -19").
3. C.B. Vagish, A.D. Kumar, K. Kumara, H.K. Vivek, N. Renuka, N.K. Lokanath, K.A. Kumar, environmentally benign synthesis of substituted pyrazoles as potent antioxidant agents, characterization and docking studies, J. Iran. Chem. Soc. 2012; 18: 479–493.
4. M. Rana, R. Arif, F.I. Khan, V. Maurya, R. Singh, M.I. Faizan, S. Yasmeen, S.H. Dar, R. Alam, A. Sahu, T. Ahmad, Rahisuddin, Pyrazoline analogs as potential anticancer agents and their apoptosis, molecular docking, MD simulation, DNA binding and antioxidant studies, Bioorg. Chem. 2021; 108: 104665.
5. Maria Shadrina, Petr A Slominsky, S.A.Limborska. Molecular mechanisms of pathogenesis of Parkinson's disease. Int rev cell mol Biol 2010, 281:229-266.
6. Jenner P. Oxidative stress in Parkinson's disease. Ann Neuron Suppl 3 2003, 53:36-38.
7. Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006, 443(7113):787-795.
8. Josue Baeza, Michael J Smallegan, John M Denu. Mechanisms and dynamics of protein acetylation in mitochondria. Trends Biochem 2016, 41(3): 231-24
9. Biedler JL, Roffler-Tarlov S, Schachner M, Freedman LS. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Research. 2012; 38: 3751–3757.
10. Presgraves SP, Ahmed T, Borwege S, Joyce JN. Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists. Neurotoxicity Research. 2014; 5: 579–598
11. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiology of Aging. 2013; 24: 197–211.
12. Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: the dual hit theory revisited. Annals of the New York Academy of Sciences. 2019; 1170: 615–622.
13. Kosanić, M., Ranković, B.R., and Dasic, M. Antioxidant and antimicrobial properties of mushrooms, Bulg. J. Agric. Sci. 2103; 19 (5): 1040–1046.
14. Tsai R, Tsai C, Liu S, Gao J, Kuo Y, Chao P, et al. Maackiain Ameliorates 6-Hydroxydopamine and SNCA Pathologies by Modulating the PINK1/Parkin Pathway in Models of Parkinson's Disease in Caenorhabditis elegans and the SH-SY5Y Cell Line. International Journal of Molecular Sciences. 2020; 21: 4455.
15. Pakrashi S, Chakraborty J, Bandyopadhyay J. Neuroprotective Role of Quercetin on Rotenone-Induced Toxicity in SH-SY5Y Cell Line Through Modulation of Apoptotic and Autophagic Pathways. Neurochemical Research. 2020; 45: 1962–1973