Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(10):258-266

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antimicrobial, Anti-Inflammatory and Anti-Parkinson's Screening of Imine Analogues through HSP90 Inhibition

M Ganga Raju*, L Saritha, Sayan Dutta Gupta and N Divya

Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India

---

ABSTRACT

Objective: Heat shock protein90 is one of a group of molecular chaperones responsible for managing protein folding and quality control in cell environment. Method: In this study an attempt is made to explore role of HSP90 in various activities like antimicrobial, anti-inflammatory, anti-Parkinson's activity. Two test compounds namely S31 and S33 belonging to class HSP90 inhibitors were studied. The antibacterial and antifungal activities were evaluated by disc-diffusion method using ciprofloxacin and fluconazole as standards respectively. The anti-inflammatory activity of the synthesized compounds was screened by carrageenan and formalin induced paw edema model using indomethacin as standard. The anti-Parkinson's activity of the compounds was screened by reserpine and haloperidol induced Parkinson's disease model using bromocriptine as standard drug. Results: From the toxicity studies, the animals were found to be safe upto a dose of 2000 mg/kg bd.wt. The results have shown that the two synthesized compounds S31 and S33 exerted significant antimicrobial, anti-inflammatory, anti-Parkinson's activity. Conclusion: These activities of the compounds might be due to the inhibition of HSP90 there by arresting the subsequent protein disaggregation and degradation. The S31 has shown significant activity over S33.

Keywords: Test compounds S31 and S33; Anti-microbial; Anti-inflammatory; Parkinson's activity; Anti-oxidant activity

---

INTRODUCTION

Heat shock proteins (HSPs) are a family of highly conserved stress proteins which can be induced by environmental stress such as heat, hypoxia, DNA damage or UV radiation to regulate cell metabolism and protect prokaryotic and eukaryotic cells from harmful exogenous stimulation. They are highly conserved ubiquitous proteins among the species which are involved in maintaining appropriate folding and conformation of other proteins and are thus referred to as molecular chaperones [1]. The heat shock response was first identified in 1962 when Ritossa described the formation of chromosome puffs in the salivary glands of the fruit fly *Drosophila bucksii* subjected to temperature elevation, sodium salicylate or dinitrophenyl [2]. In eukaryotic organisms the expression of heat shock protein messenger RNA is mediated by a family of transcription factors, called heat shock factors. Heat shock factor I (HSF I) plays a major role in heat shock response, while other members of the family are activated after prolonged stress or participate in processes such as embryonic development, or cell differentiation. Heat shock protein90 is a highly conserved and essential molecular chaperone throughout the eukaryotic lineage. HSP90 is an ATP-dependent molecular chaperone. It is one of the most abundant cytoplasmic proteins in unstressed cells, where it performs housekeeping functions, controlling the activity, intracellular disposition, and proteolytic turnover of a variety of proteins. It is required for the activation and stabilization of a wide variety of client proteins. Client proteins of

HSP90 play a central pathogenic role in human diseases including cancer, neurodegenerative diseases and viral infections [1].

MATERIALS AND METHODS

Synthesized Compounds (S31 and S33)

The test compounds synthesized (Figure 1) [3].

Dose Preparation of Test Drugs S31 and S33

The test drugs were taken and dissolved in 10% DMSO which acts as a solubilizing agent and then the volume is made up with saline. This must be done according to the concentration required.

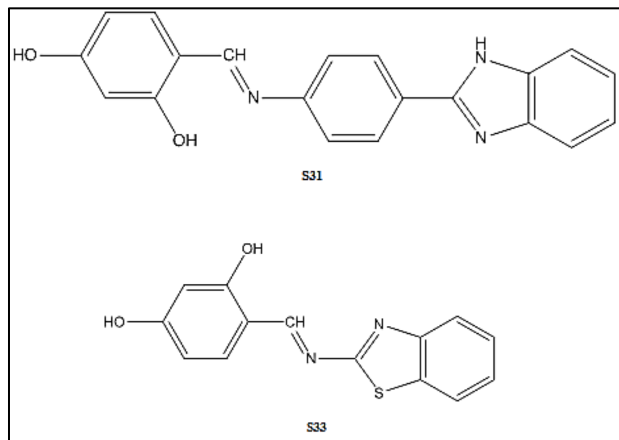

Figure 1: Synthesized compounds (S31 and S33)

Chemicals

Methanol was obtained from Crescent trading company. Reserpine was procured from Laila Impex, Vijayawada, Pvt. Ltd. The chemicals used in the present study were of analytical grade and purchased from Coral Pvt. Limited.

Experimental Animals

Albino Wistar rats (200-250 g) of either sex approximately the same age, procured from Mahaveer Enterprises, Hyderabad, India were used for the study. They were housed in polypropylene cages and fed with standard rodent pellet diet and water *ad libitum.* The animals were exposed to alternate cycle of 12 hrs of darkness and 12 hrs of light. The ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before the experiment (Reg. No.1175/PO/ERe/S/08/CPCSEA).

Acute Toxicity Studies

Acute toxicity study was conducted on compounds S31 and S33 following OECD guidelines-425. The dosage for the pharmacological studies was selected as $1/10^{th}$ of the highest dose (2000 mg/kg) administered.

Experimental Design

Antibacterial and antifungal activity:

The antimicrobial activity of the test compounds were performed using agar disc diffusion method to reveal the minimal inhibitory concentrations (MIC), i.e., the lowest concentrations of the compounds that inhibit the visible growth of the tested microorganism. Petri plates containing 20 ml of Nutrient Agar (NA) media for bacteria and Sabourand's dextrose agar (SDA) for fungi were used. After solidification of the media, ditch was made in the plates with the help of cup-borer (0.85 cm) and then the test compound was inoculated into the well. Ciprofloxacin was used as a standard for bacteria and Fluconazole was used as a positive control for fungi. Plates inoculated with bacteria incubated for 24 h at 37°C and the fungal culture was incubated for 48 h at 25°C. The inhibition zone diameters were measured in millimeters. All the tests were performed in triplicate and the average was taken as final reading [4].

Anti-inflammatory activity:

Table 1: Experimental design for carrageenan induced paw edema model (n=6)

Groups	Treatment
I	Saline + 1% carrageenan
II	100 mg/kg bd. wt of test drug S31+1% carrageenan
III	200 mg/kg bd. wt of test drug S31+1% carrageenan
IV	100 mg/kg bd. wt of test drug S33+1% carrageenan
V	200 mg/kg bd. wt of test drug S33+1% carrageenan
VI	10 mg/kg bd. wt of standard drug indomethacin+1% carrageenan

Induction of inflammation:

The Wistar rats weighing 200-250 g were selected and numbered them and divided the animals into six groups each group containing six rats (n=6). Make a mark on the both hind paws (right and left) of all the animals just beyond tibia-tarsal junction, so that every time the paw dipped in the mercury column upto the fixed mark to ensure constant paw volume (Tables 1 and 2). Note the initial paw volume (both right and left) of all the group of animals by mercury displacement method. Group I animals receives Saline. Group II and III animals receives test compound S31 at a doses of 100 and 200 mg/kg bd.wt. Group IV and V receives test compound S33 at a doses of 100 and 200 mg/kg bd.wt are administered through *orally* and finally the group VI receives standard drug indomethacin orally. After 30 minutes, inject 0.1 ml of 1% (w/v) carrageenan in the sub plantar region of the left paw of all groups of animals. The right will serve as reference non-inflamed paw for comparison [5]. Note the paw volume of both legs of all groups of animals at 1 h, 2 h, 3 h and 4 h after formalin challenge [5].

Table 2: Experimental design for formalin induced paw edema model (n=6)

Groups	Treatment
I	Saline+1% formalin
II	100 mg/kg bd. wt of test drug S31+1% formalin
III	200 mg/ kg bd. wt of test drug S31+1% formalin
IV	100 mg/kg bd. wt of test drug S33+1% formalin
V	200 mg/kg bd. wt of test drug S33+1% formalin
VI	10 mg/kg bd. wt of standard drug indomethacin+1% formalin

Anti-Parkinson's activity:

Table 3: Experimental design for haloperidol induced Parkinson's disease model (n=6)

Groups	Treatment
I	Saline (normal control)
II	Saline+ 2 mg/kg haloperidol
III	100 mg/kg bd. wt of test drug S31+2 mg/kg haloperidol
IV	200 mg/kg bd. wt of test drug S31+2 mg/kg haloperidol
V	100 mg/kg bd. wt of test drug S33+2 mg/kg haloperidol
VI	200 mg/kg bd. wt of test drug S33+2 mg/kg haloperidol
VII	2.5 mg/kg bd. wt of standard drug bromocriptine+2 mg/kg haloperidol

Anti-Parkinson's activity was done by using haloperidol induced Parkinson's disease model. Wister albino rats weighing 200-250 g were selected and divided into seven groups (Tables 3 and 4). Group I and II receives saline and group III and IV receives test compound S31 at a dose of 100 and 200 mg/kg bd.wt and group V and VI receives test compound S33 at a dose of 100 and 200 mg/kg bd.wt through *oral* route and finally group VII receives standard drug bromocriptine at a dose of 2.5 mg/kg bd.wt through *s.c* route. After 1h all animals receive haloperidol except first group through *i.p* route upto 8 days. Parameters were observed on 8th day for all the rats.

Table 4: Experimental design for reserpine induced Parkinson's disease

Groups	Treatment
I	Saline (normal control)
II	Saline+5 mg/kg reserpine
III	100 mg/kg bd. wt of test drug S31+5 mg/kg reserpine
IV	200 mg/kg bd. wt of test drug S31+5 mg/kg reserpine
V	100 mg/kg bd. wt of test drug S33+5 mg/kg reserpine
VI	200 mg/kg bd. wt of test drug S33+5 mg/kg reserpine
VII	2.5 mg/kg bd. wt of standard drug bromocriptine+5 mg/kg reserpine

Procedure:
Anti-Parkinson's activity was done by using reserpine induced Parkinson's model. Swiss albino mice are weighing 25-30 g were selected and divided into seven groups. All groups of animals receive reserpine 5 mg/kg bd. wt. through *i.p* 24 h before administration of test drugs. After 24 h group III and IV receives test compound S31 at a dose of 100 and 200 mg/kg bd.wt and group V and VI receives test compound S33 at a dose of 100 and 200 mg/kg bd.wt and group VII receives standard drug bromocriptine at a dose 2.5 mg/kg bd. wt is administered through *s.c* route. After 1h parameters were observed [6].

Parameters to be determined:
Resting tremor: Tremor of the whole body was evaluated in mice/rats utilizing the rating scale
- 0 – No tremor
- 1 – Occasional isolated twitches
- 2 – Moderate tremor
- 3-5 – Continuous tremor

Ptosis:
- 4 – Eyes completely closed
- 2 – Half-open eyes
- 0 – Wide-open eyes
- 1 and 3 – Indicating intermediate values

Bradykinesia: The mice/rats were held by the tail so that he is standing by his forelimbs and moving on its own. The number of steps taken with both forelimbs was recorded for 30 seconds.

Catalepsy:
1. Catalepsy was evaluated using the bar test in which, the mice/rats were placed in half rearing position with both the front paws on a horizontal bar, 9 cm above and parallel to the base.
2. Mice were observed with a stopwatch to note the time of removal of one paw from the bar. The maximum cut off time for observation was fixed at 180 sec.

Righting reflex: The righting reflex was evaluated by turning the mice/rats onto back five times.
Righting reflex was scored as follows:
- 0 – No impairment
- 1 – On side one to two times
- 2 – On side three to four times
- 3 – On side five times
- 4 – On back one to two times
- 5 – On back three to four times
- 6 – On back five time
- 7 – Righting response absent when on back

Muscular rigidity: The parameter was measured by using Rota-rod apparatus. First weigh the animals and number them and then turn on the Rota-rod and select an appropriate speed (20-25 rpm). Place the animal one by one on the rotating rod. Note down the fall of time when the mice falls from the rotating rod. A normal (untreated) mouse generally falls off within 3-5 minutes. Repeat the procedure after 30 minutes drug administration.

Locomotor: The parameter was measured by using Actophotometer apparatus. First weigh the animals and number them and then turn on the equipment (check and make sure that all the photo cells are working for accurate recording) and place individually each mice/rats in the activity cage for 10 min. Note the basal activity score of all the animals. Re-test the animals after 30 minutes of drug administration for the duration of 10 minutes.

Swim test: Each mice was introduced individually into a pool (45 cm long, 22 cm wide diameter and 20 cm high) filled with 10 cm deep water (21°C-23°C). The mice were allowed to swim upto 5 minutes. The swimming time was recorded until the mice/rats stop swimming and just float in water with its head out of the water level. The same procedure was repeated with all the animals after 30 minutes of drug administration [7,8].

In vivo Antioxidant Studies

Antioxidant studies are performed using determination of hydrogen peroxide scavenging assay and reducing power assay [9].

Histopathological Studies

A portion of brain of normal control, haloperidol control, test drugs S31 (200 mg/kg), S33 (200 mg/kg) and standard bromocriptine were stored in containers for 12 hours in 10% formalin solution and subjected to histopathological studies. Observed microscopically for histopathological changes i.e., normal brain, damaged and recovered brain was studied and compared. The results were shown in Figure 2.

Standard Bromocriptine 2.5 mg/kg

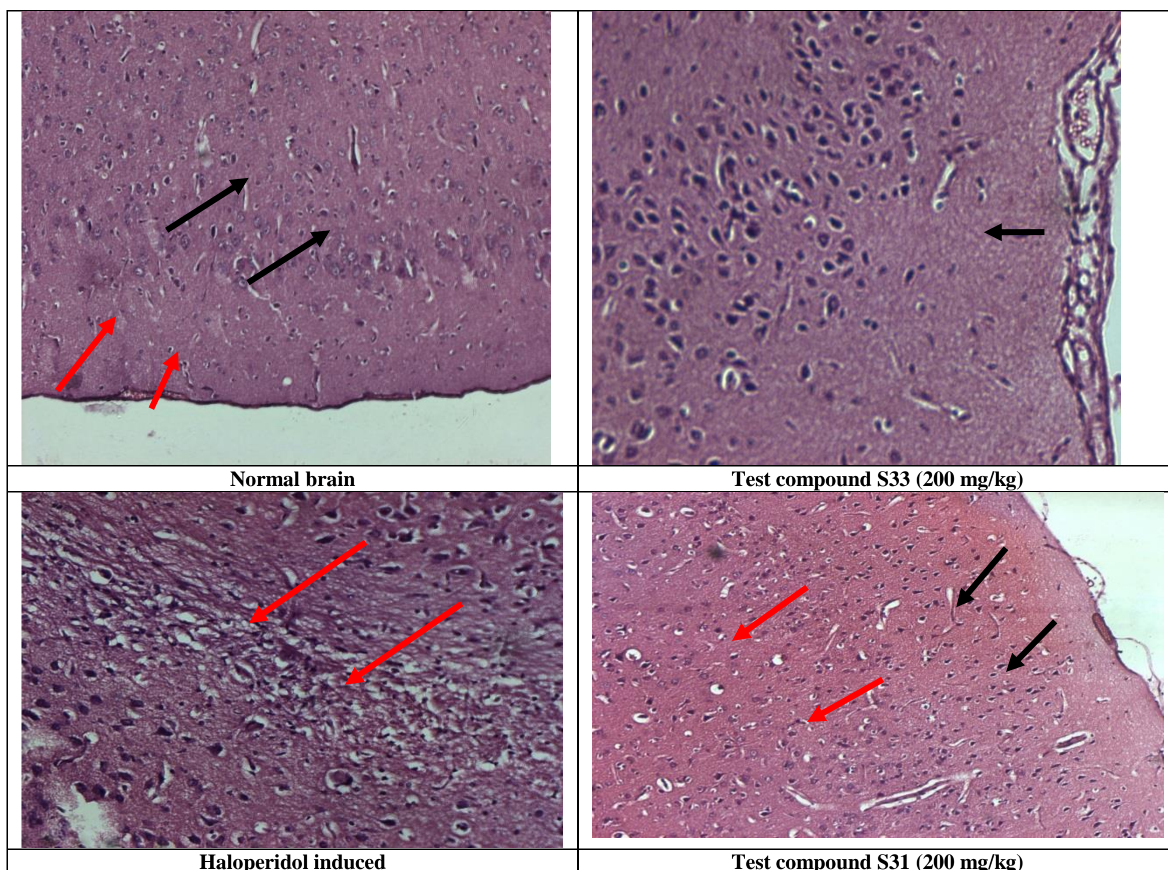

Figure 2: Histopathological study of brain in haloperidol induced model

Statistical Analysis

All the values were expressed as mean ±SEM. The data's were statistically analyzed by one way ANOVA followed by Dunnett's t-test and values p<0.05 was considered to be significant.

RESULTS

Acute Toxicity Studies

The test drugs S31 and S33 were showed safe upto a dose of 2000 mg/kg, bd.wt with no signs of mortality, hence the dose of 100 mg/kg and 200 mg/kg were considered for the study.

Antibacterial Activity

The test drugs S31 and S33 have shown MIC at two concentrations, 50 µg/ml and 500 µg/ml, where as the standard drug ciprofloxacin has shown MIC at concentration less than 10 µg/ml. Values are expressed as mean±SEM of three replicate analysis (Table 5).

Table 5: Antibacterial activity of test compounds S31 and S33

Microorganisms	S31		S33		Ciprofloxacin	
	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)
Bacillus subtilis	50	17.00 ± 0.13	500	25.00 ± 0.04	<10	26.66 ± 0.45
Bacillus pumilis	50	16.66 ± 0.20	500	20.00 ± 0.05	<10	25.00 ± 0.35
Escherichia coli	50	16.33 ± 0.88	500	23.66 ± 0.04	<10	26.33 ± 0.64
Pseudomonas aureginosa	50	16.33 ± 0.66	500	25.00 ± 0.04	<10	27.36 ± 0.68

Antifungal Activity

The test drugs S31 and S33 have shown MIC at concentration 100 µg/ml where as the standard drug fluconazole has shown MIC at concentration less than 10 µg/ml (Table 6).

Table 6: Antifungal activity of test compounds S31 and S33

Compounds	*Candida albicans*		*Aspergillus fumigates*	
	MIC (µg/ml)	Zone of inhibition (mm)	MIC (µg/ml)	Zone of inhibition (mm)
S31	100	21.33 ± 0.60	100	25.00 ± 1.30
S33	100	20.00 ± 0.55	100	24.33 ± 0.80
Fluconazole	<10	28.33 ± 1.00	<10	28.66 ± 1.20

Values are expressed as mean ± SEM of three replicate analysis.

Anti-inflammatory Activity

Carrageenan induced anti-inflammatory activity:

The anti-inflammatory activity was performed using carrageenan induced paw oedema model (Table 7). The paw thickness at 1 h, 2 h, 3 h and 4 h and the percentage reduction in paw oedema at the end of third hour is calculated. The two test drugs S31 and S33 have shown significant reduction in paw edema when compared to control and standard groups.

Table 7: Anti-inflammatory activity of test compounds S31 and S33 by carrageenan induced paw edema model

Compounds	Change in paw edema (mL)				% inhibition in paw edema at 3 h
	1h	2 h	3 h	4 h	
Carrageenan induced	2.3 ± 0.04	4.0 ± 0.057	6.0 ± 0.05	24 ± 1.0	-
S31 100 mg/kg bd.wt	6.6 ± 0.40	6.0 ± 0.040	3.3 ± 0.056	2.3 ± 0.05	42.0 ± 1.0[a], [**]
S31 200 mg/kg bd.wt	6.6 ± 0.40	5.0 ± 0.040	2.0 ± 0.040	1.6 ± 0.50	65.0 ± 2.0[a], [**]
S33 100 mg/kg bd.wt	7.6 ± 0.05	6.3 ± 0.005	2.6 ± 0.003	2.0 ± 0.051	59.0 ± 1.4[b], [**]
S33 200 mg/kg bd.wt	7.1 ± 0.05	4.6 ± 0.050	2.3 ± 0.002	2.3 ± 0.05	61.0 ± 2.3[a, **]
Indomethacin 10 mg/kg bd. Wt	6.3 ± 0.04	4.3 ± 0.040	1.6 ± 0.005	1.6 ± 0.04	70.57 ± 1.5[a]

Values are expressed as mean ± SEM; all the groups were compared with control and standard. Significant values are control (a = p<0.001, b= p<0.05), standard ([**] = p<0.001, [*] = p<0.05) using Dunnett's-test

Formalin induced anti-inflammatory activity:

The anti-inflammatory activity was performed using formalin induced paw oedema model. The paw thickness at 1 h, 2 h, 3 h and 4 h and the percentage reduction in paw oedema at the end of third hour were calculated (Table 8). The

test drugs S31 and S33 have shown significant reduction in paw edema when compared to control and standard groups.

Table 8: Anti-inflammatory activity of test compounds S31 and S33 by formalin induced paw edema model

Groups	Change in paw edema (mL)				% inhibition in paw edema at 3 h
	1 h	2 h	3 h	4 h	
Formalin Induced	1.6 ± 0.04	3.0 ± 0.6	5.0 ± 0.7	7.3 ± 0.5	_
S31 100 mg/kg bd.wt	7.3 ± 0.80	6.3 ± 0.8	4.3 ± 0.04	2.6 ± 0.05	$24.33 \pm 0.5^{a, *}$
S31 200 mg/kg bd.wt	6.6 ± 0.60	6.0 ± 0.79	1.6 ± 0.05	2.0 ± 0.05	$64.4 \pm 0.6^{a, **}$
S33 100 mg/kg bd.wt	7.0 ± 0.75	6.0 ± 0.79	3.0 ± 0.06	2.3 ± 0.06	$43 \pm 0.5^{b, *}$
S33 100 mg/kg bd.wt	6.6 ± 0.70	5.0 ± 0.5	2.3 ± 0.05	2.0 ± 0.05	$50 \pm 0.4^{a, **}$
Indomethacin 10 mg/kg bd.wt	6.3 ± 0.8	4.0 ± 0.04	2.0 ± 0.05	1.6 ± 0.04	65 ± 0.6

Values are expressed as mean±SEM, all the groups were compared with control and standard. Significant values are control (a = p<0.001, b= p<0.05), standard (** = p<0.001, * = p<0.05). using Dunnett's-test

Anti-Parkinson's Activity

Haloperidol induced Parkinson's disease model:

Anti-Parkinson's activity was performed by using haloperidol induced Parkinson's disease model. The parameters were observed. The test drugs of S31 at a doses of 100 and 200 mg/kg bd. wt and S33 at a doses of 100 and 200 mg/kg bd.wt has shown significant activity in various parameters compared with control, haloperidol induced and standard bromocriptine (Tables 9 and 10).

Table 9: Anti-Parkinson's activity of test drugs S31 and S33 by haloperidol induced Parkinson's disease model

Compounds	1. Ptosis	2. Tremors	3. Righting reflex	4. Bradykinesia
Control	--	--	--	8.0 ± 0.5
Haloperidol Induced	$4.0 \pm 0.7^{a, A}$	$3.0 \pm 0.03^{a, A}$	$7.0 \pm 0.2^{a, A}$	$3.3 \pm 0.3^{a, A}$
S31 100 mg/kg bd.wt	$2.3 \pm 0.5^{a,*,B}$	$1.6 \pm 0.3^{a,*,A}$	$2.3 \pm 0.3^{a,**,A}$	$6.0 \pm 0.5^{a,**,B}$
S31 100 mg/kg bd.wt	$0.6 \pm 0.2^{a,**,A}$	$2.5 \pm 0.3^{a,**,A}$	$4.3 \pm 0.2^{b,**,B}$	$6.6 \pm 0.6^{a,**,A}$
S33 100 mg/kg bd. Wt	$2.3 \pm 0.7^{a,**,B}$	$2.0 \pm 0.01^{a,*,B}$	$2.6 \pm 0.4^{a,**,A}$	$5.6 \pm 0.3^{a,**,B}$
S33 100 mg/kg bd. Wt	$1.3 \pm 0.4^{a,**,A}$	$2.0 \pm 0.3^{b,**,A}$	$5.0 \pm 0.3^{a,**,A}$	$6.6 \pm 0.3^{a,**,A}$
Bromocriptine 2.5 mg/kg bd. wt	$0.3 \pm 0.01^{a,**}$	$1.5 \pm 0.3^{a,**}$	$5.6 \pm 0.4^{b,,**}$	$7.3 \pm 0.3^{a,**}$

Values are expressed as mean±SEM; all the groups were compared with control, haloperidol induced and standard. Significant values are control (a = p<0.001, b= p<0.05) haloperidol induced (** = p<0.001, * = p<0.05) Standard (A = p<0.01, B = p<0.05) using Dunnett's t-test

Table 10: Anti-Parkinson's activity of test compounds S31 and S33 by haloperidol induced Parkinson's disease model

Compounds	5. Swim test	6. Locomotor	7. Catalepsy	8.Muscular rigidity
Control	$210 \pm 9.0^{**,A}$	$183 \pm 5.0^{*,A}$	$116 \pm 8.0^{**,B}$	$153 \pm 8.0^{b,**,A}$
Haloperidol Induced	$46 \pm 4^{a,**,A}$	$37.6 \pm 4.4^{a,**,B}$	$18.3 \pm 1.2^{b,**,A}$	$18.3 \pm 1.5^{b,**,A}$
S31 100 mg/kg bd.wt	$76 \pm 5.9^{a,*,A}$	$73.3 \pm 3.3^{a,*,A}$	$46 \pm 3.3^{a,*,B}$	$63.3 \pm 0.5^{b,**,A}$
S31 200 mg/kg bd.wt	$146 \pm 7.2^{a,**,B}$	$146 \pm 4.0^{a,**,A}$	$103 \pm 7.5^{b,**,A}$	$103 \pm 4.5^{b,**,A}$
S33 100 mg/kg bd.wt	$53 \pm 4.0^{b,**,A}$	$86.6 \pm 3.8^{a,*,B}$	$40 \pm 3.5^{a,**,B}$	$53 \pm 2.8^{b,**,A}$
S33 200 mg/kg bd.wt	$136 \pm 7.0^{a,**,A}$	$183.3 \pm 4.0^{a,**,A}$	$83 \pm 4.5^{a,**,A}$	$90 \pm 3.8^{b,**,A}$
Bromocriptine 2.5 mg/kg bd. Wt	$186.6 \pm 8.5^{a,**}$	$170 \pm 5.0^{a,**}$	$106 \pm 7.8^{a,**}$	$143 \pm 7.8^{b,**,}$

Values are expressed as mean±SEM; all the groups were compared with control, haloperidol induced and standard. Significant values are control (a = p<0.001, b= p<0.05) haloperidol induced (** = p<0.001, * = p<0.05) Standard (A = p<0.01, B = p<0.05) using Dunnett's t-test

Reserpine induced Parkinson's disease model:

Anti-Parkinson's activity was performed by using reserpine induced Parkinson's disease model. The parameters were observed after 24 h. The test drugs S31 at a doses of 100 and 200 mg/kg bd.wt and S33 at a doses of 100 and 200 mg/kg bd.wt has shown significant activity in various parameters compared with control (Tables 11 and 12) reserpine induced and standard drug bromocriptine.

Table 11: Anti-Parkinson's activity of test compounds S31 and S33 by reserpine induced Parkinson's disease model

Compounds	1. Ptosis	2. Tremors	3. Righting reflex	4. Bradykinesia
Control	--	--	--	15 ± 1.0
Reserpine Induced	$3.3 \pm 0.6^{a,A}$	$2.6 \pm 0.3^{a,A}$	$7.0 \pm 0.2^{a, A}$	$4 \pm 0.5^{a,A}$
S31 100 mg/kg bd.wt	$2.3 \pm 0.5^{a,*,B}$	$2.0 \pm 0.5^{a,*,A}$	$2.0 \pm 0.22^{a,**,A}$	$8 \pm 0.5^{a,**,B}$
S31 200 mg/kg bd.wt	$0.6 \pm 0.2^{a,**,A}$	$1.0 \pm 0.5^{a,**,B}$	$5.0 \pm 0.2^{b,**,B}$	$9 \pm 0.5^{a,**,A}$
S33 100mg/kg bd. Wt	$2.0 \pm 0.5^{a,**,A}$	$2.3 \pm 0.3^{a,*,A}$	$1.6 \pm 0.21^{a,*,A}$	$8.3 \pm 1.8^{a,**,A}$
S33 200 mg/kg bd. Wt	$1.6 \pm 0.3^{a,**,A}$	$1.0 \pm 0.5^{b,**,A}$	$4.3 \pm 0.2^{a,**,B}$	$11.6 \pm 1.5^{a,**,A}$

Values are expressed as mean±SEM; all the groups were compared with control, haloperidol induced and standard. Significant values are control (a = p<0.001, b= p<0.05) haloperidol induced (** = p<0.001, * = p<0.05) Standard (A = p<0.01, B = p<0.05) using Dunnett's t-test

Table 12: Anti-Parkinson's activity of test compounds S31 and S33 by reserpine induced Parkinson's disease model

Compounds	5. Swim test	6. Locomotor	7. Catalepsy	8. Muscular rigidity
Control	$193 \pm 8.0^{**,A}$	$216 \pm 10^{*,A}$	$76.6 \pm 6.3^{**,B}$	$133 \pm 7.0^{**,A}$
Reserpine Induced	$50 \pm 4.0^{a,A}$	$35 \pm 8.6^{a,B}$	$11.6 \pm 1.5^{a,A}$	$18 \pm 1.5^{a, A}$
S31 100 mg/kg bd. wt	$70 \pm 5.7^{a,*,A}$	$93.3 \pm 3.3^{a,**,A}$	$40 \pm 3.5^{a,*,A}$	$56 \pm 3.5^{a,**,B}$
S31 200 mg/kg bd. Wt	$126 \pm 7.0^{a,**,B}$	$166.6 \pm 10^{a,**,B}$	$70 \pm 6.6^{a,*,A}$	$90 \pm 4.4^{a,**,B}$
S33 100 mg/kg bd. Wt	$60 \pm 3.3^{b,**,A}$	$90 \pm 5.7^{a,*,A}$	$46 \pm 3.3^{a,**,A}$	$46 \pm 2.5^{a,**,A}$
S33 200 mg/kg bd. Wt	$110 \pm 6.0^{a,**,A}$	$203.3 \pm 8^{a,**,B}$	$60 \pm 7.6^{a,**,B}$	$83 \pm 3.7^{a,**,A}$
Bromocriptine 2.5 mg/kg bd. Wt	$173 \pm 8.0^{a,**}$	$210 \pm 10^{a,**}$	$73.3 \pm 6.0^{a,**}$	$113 \pm 6.5^{b,**}$

Values are expressed as mean±SEM; all the groups were compared with control, haloperidol induced and standard. Significant values are control (a = p<0.001, b= p<0.05) haloperidol induced (** = p<0.001, * = p<0.05) Standard (A = p<0.01, B = p<0.05) using Dunnett's t-test

DISCUSSION

Antibacterial Activity

Temperature protein G (HtpG), a bacterial heat shock protein 90 helps in resting variations in temperature and also stress conditions by supporting *de novo* protein folding hence it is essential for the proper survival of bacteria in varied conditions. In the present investigations the results clearly depicted significant dose-dependent antibacterial activity of the test compounds S31 and 33 when compared with standard ciprofloxacin. This activity of the test compounds might be due to inhibition of HtpG leading to intolerance to temperature variations and improper *de novo* protein folding that causes lysis of bacterial cell [10].

Antifungal Activity

HSP90 is naturally abundant in fungal cells and is induced to even greater levels by heat shock and other protective stresses. HSP90 levels are regulated at transcriptional and post-transcriptional levels. Fungi uses fungal HSP90-calcineurin pathway in cell wall synthesis, ion homeostasis and other functions. Calcineurin function is governed by HSP90 as it is one client protein of HSP90. The antifungal activity of test drugs might be due to inhibition of fungal HSP90 [11].

Anti-inflammatory Activity

Endothelial nitric oxide synthase (eNOS) is considered to be the primary source of NO in inflammation. HSP90 associates with eNOS and stimulate production of nitric oxide. As eNOS is one of the client proteins of HSP90, its ability to produce NO depends upon HSP90. So when HSP90 is inhibited eNOS is down-regulated and retards the production of NO. The anti-inflammatory activity of the two test drugs S31 and S33 might be due to down-regulation of eNOS by inhibiting HSP90 [12].

Anti-Parkinson's Activity

In Parkinson's disease there is inhibition of HSP90 which induces HSF-1. This activated HSF-1 produces mainly two chaperones Hsp70 and Hsp40. These activated chaperone cause disaggregation, degradation and misfolding of various proteins. The anti-Parkinson's activity of the two test drugs have might be due to inhibition of HSP90 [13].

CONCLUSION

These results suggested that synthesized compounds (S31 and S33) possess significant antimicrobial, anti-inflammatory and anti-Parkinson's activity.

REFERENCES

[1] KC Kregel. *J Appl Physiol*. **2002**, 92, 2177-2186.
[2] F Ritossa. *Experientia J*. **1962**, 18, 571-573.
[3] SD Gupta; B Revathi; G Mazaira; MD Galigniana; CVS Subrahmanyam; NL Gowrishanker; NM Raghavendra. *Bioorg Chem*. **2015**, 97-105.
[4] A Zovko; MV Gabric; K Sepcic; F Pohleven; D Jaklic; NG Cimerman; Z Lu; RE Ebel; WE Houssen; I Mancini; A Defant; M Jaspars; T Turk. *Int Biodeterioration Biodeg*. **2012,** 68, 71- 77.
[5] UP Singh; P Kumar; M Manish; S Thenmozhi; BR Balakrishnan. *Ancient Sci Life*. **2013**, 32(3), 150-155.
[6] S Manikandaselvi; R Mahalakshmi; R Thinagarbabu; AR Angumeenal. *Int J Pharm Tech Res*. **2012**, 4(2), 669-675.
[7] JL Calderon; R Bolanos. *Rivista Neuropsicol Neurosiquiatria Y Neurociencias*. **2011**, 5(11), 49-61.
[8] MT Tadaiesky; R Andreatini; MABF Vital. *Eur J Pharmacol*. **2006**, 535, 199-207.
[9] MD Ali Ebrahimzadeh; SM Nabavi; SF Nabavi; F Bahramian; AR Bekhradnia. *Pak J Pharm Sci.* **2010**, 23 (1), 29-34.
[10] H Nakamoto; K Fujita; A Ohtaki; S Watanabe; S Narumi; T Maruyama; E Suenaga; TS Misono; PKR Kumar; P Goloubinoff; H Yoshikawa. *J Biological Chem.* **2014**, 1-23.
[11] LE Cowen. *Nature Reviews Microbiol J*. **2008**, 187-198.
[12] JL Lowry; V Brovkovych; Y Zhang; RA Skidgel. *J Biological Chem*. **2012**, 288(6), 4174-4193.
[13] W Luo; W Sun; T Taldone; A Rodina; G Chiosis. *Molecular Neurodegen.* **2010**, 5(24), 45-47.