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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF N-(4-{[2-(2-OXO-1,2-DIHYDRO-3H-INDOL-3-LIDENE)HYDRAZINYL] SULFONYL}PHENYL)ACETAMIDE

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ABSTRACT

A series of *N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-lidene)hydrazinyl] sulfonyl} phenyl) acetamides were synthesized from Isatins and were evaluated for antimicrobial and antioxidant activities.

KEYWORDS: Isatin, Antimicrobial, antioxidant, cup plate method, DPPH method.

INTRODUCTION

Indole 2, 3-dione (Isatin) has occupied a unique position in medicinal chemistry and is also an interesting moiety because of its use in many pharmaceutical, biological and analytical applications. Isatin has been known for about 150 years to exhibit biological activity in mammals. The synthetic versatility of isatin analogs at C-2, C-3, and *N* position has lead to a wide variety of pharmacological response including anti-bacterial, antifungal[1-3], antioxidant[4], analgesics[5-9], antineoplastics[10], antifertility[11], sedatives and hypnotics[12-19], anticonvulsant[20], anxiolytics[21],monoamino oxidase inhibitors[22] Laxatives[23], Diuretics[24-26], antihypoxic agents[27-30], anaphylactic shock[31-34], antitubercular.[35-38] Isatin and its analogs act on a vast number of biological targets[39-40] including proteases, caspases, kinases, reverse transcriptase, extracellular signal regulated protein kinase (ERK). The 2-oxoindole derivatives SU-5416 (semaxanib) and SU- 11248 (sunitinib) reportedly possess tyrosine kinase inhibitory and antiangiogenic properties. Based on the versatility of the isatin moiety and its importance in the development of antimicrobials, it was thought worthwhile to design and synthesize some novel *N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-lidene) hydrazinyl] sulfonyl}phenyl)acetamides.

Synthesis of Indole-2,3-diones (Isatins)

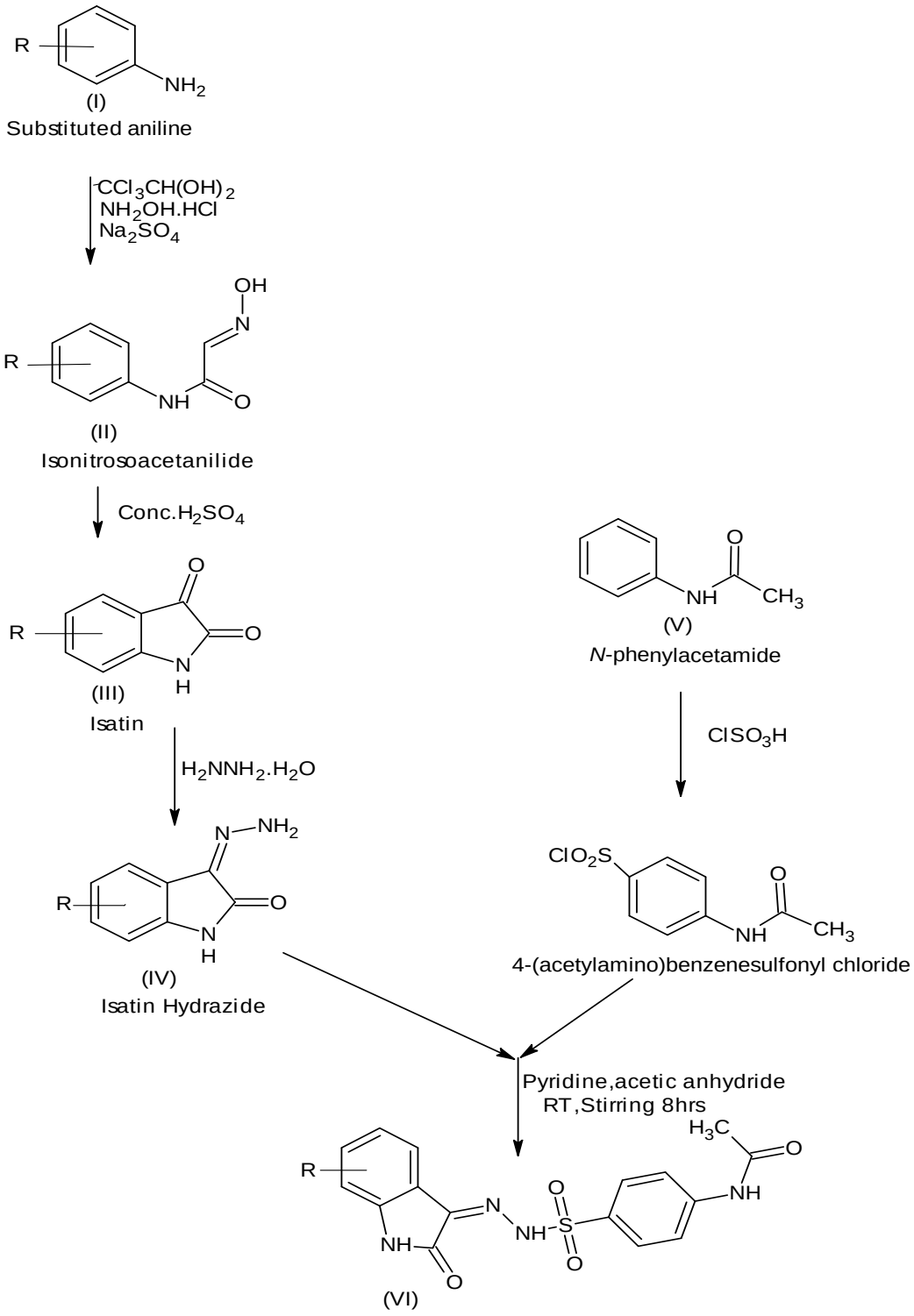
a) Isonitrosoacetanilide – General Procedure

In a 5 lit. R.B. flask were placed chloral hydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300gm) followed by a solution of an appropriate aromatic amine in 300ml of water and concentrated hydrochloric acid (0.52mol). Finally, a solution of hydroxylamine HCl (1.58 mol) in 500ml of water was added. The contents of the flask were heated over a wire-gauge by a Mecker burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilide started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent(s).

b) Indole-2,3-diones – General Procedure

Sulfuric acid (600g, d:1.84, 326 ml) was warmed at 50^{o}C in a one litre RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (0.46 mol) was added at such a rate so as to maintain the temperature between 60^{o}C to 70^{o}C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80^{o}C and maintained at that temperature for 10 minutes, to complete the reaction. Then the reaction mixture was cooled to room temperature and poured onto crushed ice (2.5 kg) while stirring.After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried.

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.



N-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazinyl]sulfonyl}phenyl)acetamide

a) R=H,
b) R=5-Cl,
c) R=5-NO_2,
d) R=5-CH_3,
e) R=5-F,
f) R=7-COOH,
g) R=6-Br.

Synthesis of isatin hydrazide

Isatin was dissolved in methanol. To this solution was added 2 to 3 ml of hydrazine hydrate. Then add 3 drops of glacial acetic acid. Reflux it for 2hrs. After the reaction time is over, transfer it to beaker with crushed ice. Precipitate appears at the bottom. Compounds were purified by recrystallization from methanol and were confirmed by TLC.

Synthesis of p-Acetamido benzene sulfonyl chloride

Dry acetanilide 25g (0.185 mol) in a 500ml round bottom flask and add 63ml (1 mol) of chlorosulphonic acid was added drop wise with a dropper with continuous shaking. After complete addition reflux the reaction mixture for one hour on a water bath. Cool to room temperature and pour the reaction mixture onto crushed ice. Filter the obtained precipitate and wash it with water and dry the product. The product can be used as such in the next step.

Synthesis of *N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3- ylidene) hydrazinyl] sulfonyl} phenyl)acetamide

Isatin hydrazides 8.5gm(5M) was added into 250ml round bottom flask with dry pyridine and dissolved. To this mixture p-acetamido benzene sulfonyl chloride 11.65 gm(5M)was added and stirred using a magnertic stirrer for 8hrs at room temperature. The completion of the reaction was monitored by TLC. After the reaction is over, precipitate appears at the bottom. Keep it aside for half n hour. Filter the precipitate. and recrystalised with ethanol. (pyridine acts as catalyst, solvent, and also to neutralise the released HCl from the reaction).

Antibacterial activity by cup plate method

The antibacterial activity of synthesized compounds was conducted against three representative Gram-positive organisms viz. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* and Gram-negative organisms viz. *Escherichia coli* (MTCC 443), *Pseudomonas Aeruginosa* (MTCC 741), and *Klebsiella pneumoniae* (MTCC 618). Penicillin and Streptomycin was employed as standard to compare the results.

Culture medium: Nutrient broth was used for the preparation of inoculum of the bacteria and nutrient agar was used for the screening method.

The test organisms were subculture using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at $37^{o}C \pm$

1°C for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacteria inoculum was prepared by transferring a loopful of stock culture to nutrient broth (100 ml) in conical flasks (250 ml). The flasks were incubated at 37°C ± 1°C for 48 hours before the experimentation.

Solutions of the test compounds were prepared by dissolving 10 mg each in 1% sodium hydroxide (10 ml, AnalaR grade). A reference standard for both gram-positive and gram-negative bacteria was made by dissolving accurately weighed quantity of ampicillin sodium in sterile distilled water, separately.

The nutrient agar medium was sterilized by autoclaving at 121°C (15 lb/sq. inch) for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot-air oven at 160°, for an hour. Into each sterilized petriplate (10 cm diameter), about 27 ml of molten nutrient agar medium was poured and inoculated with the respective strain of bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) was transferred asceptically. The plates were left at room temperature to allow the solidification. In each plate, three cups of 6 mm diameter were made with sterile borer. Then 0.1 ml of the test solution was added to the respective cups asceptically and labelled, accordingly.

The plates were kept undisturbed for atleast 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. After incubation of the plates at 37°± 1°C for 24 hours, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of 1% sodium hydroxide to observe the solvent effects.

Anti Fungal Activity

In vitro antifungal activity of the newly synthesized compounds was studied against the fungal strains, *Candida. albicans* (MTCC 227) by Agar Well Diffusion Method.

The ready-made Potatoes were autoclaved at pressure of 15 lb/inc^2 for 20 min. Agar well bioassay was employed for testing antifungal activity**.** The medium was poured into sterile Petri dishes under aseptic conditions in a laminar air flow chamber. When the medium in the plates solidified, 0.5 mL of (week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by

dissolving the compound in DMSO and different concentrations were made. After inoculation, wells were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each well different concentrations of test solutions were added. Controls were maintained. The treated and the controls were kept at 27^0 C for 48 h. Inhibition zones were measured and the diameter was calculated in millimetre. Three to four replicates were maintained for each treatment. The results are presented in Tables.

RESULTS AND DISCUSSION

Table 1: Physical data of *N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) hydrazinyl] sulfonyl}phenyl)acetamide.

compound	Substitution	Molecular Formula	Mol. Wt.	Melting Point	% Yield
(VI_a)	H	$C_{16}H_{14}N_4O_4S$	358.37	182-187°C	73
(VI_b)	5-Cl	$C_{16}H_{13}ClN_4O_4S$	392.81	208-216°C	65
(VI_c)	5-NO_2	$C_{16}H_{13}N_5O_6S$	403.36	227-232°C	54
(VI_d)	5-CH_3	$C_{17}H_{16}N_4O_4S$	372.39	192-195°C	49
(VI_e)	5-F	$C_{16}H_{13}FN_4O_4S$	376.36	204-209°C	61
(VI_f)	7-COOH	$C_{17}H_{14}N_4O_6S$	402.38	241-247°C	53
(VI_g)	6-Br	$C_{16}H_{13}BrN_4O_4S$	430.43	252-254°C	41

Spectral data of compound (VI a)

IR (KBr, cm-1): 3570(N-H str), 3176(N-H), 2912(C-H str), 2096(C-S str), 1683(C=Ostr), 1174 (S=O str).

^{1}H NMR (DMSO-d6, 400 MHz) δ = 11.15 (s, 1H, NH),9.35(s,1H,NH), 8.1(s, 1H, NH), 7.93(d,2H, C-H), 7.791(d, 2H, C-H), 7.361-6.821(m,4H,C-H), 2.031(s,3H,C-H).

Mass spectrum (ESI): molecular ion (M+H) + peak was observed at 359 m/z (100%).

Spectral data of compound (VI b)

IR (KBr, cm-1): 3311(N-H str), 3125 (N-H str), 2970 (C-H str), 2110 (C-S str), 1731 (C=O str), 1674 (C=N str), 1240 (S=O str),1011 (C-Cl str).

• **1H NMR (DMSO-d6, 400 MHz)** δ = 11.19 (s, 1H, NH), 9.32(s, 1H, NH), 8.13(s 1H, NH), 7.92(d, 2H,Ar-H), 7.79(d, 2H, Ar-H), 7.49(s, 1H, Ar-H), 7.38(d,1H,Ar-H), 7.23 (d,1H,Ar-H), 2.023 (s,3H, CH3).

Antibacterial activity of substituted*N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazinyl]sulfonyl}phenyl)acetamide at different concentrations in gm +ve and gm-ve organisms at 400µg/ml

S.NO	*Compound*	*E.Coli*	*Pseudomonas*	*S.aureus*	*B.Subtilis*
1	**(VIa)**	6mm	5.3mm	11mm	12mm
2	**(VIb)**	15mm	17mm	17mm	19mm
3	**(VIc)**	2.5mm	4.0mm	7mm	8mm
4	**(VId)**	5.0mm	4.7mm	11mm	9mm
5	**(VIe)**	19mm	21mm	14mm	16mm
6	**(VIf)**	6mm	4.5mm	6mm	6.4mm
7	**(VIg)**	7.5mm	7mm	6.8mm	7.0mm
8	Ciprofloxacin	1.52mm	28mm	46mm	47mm

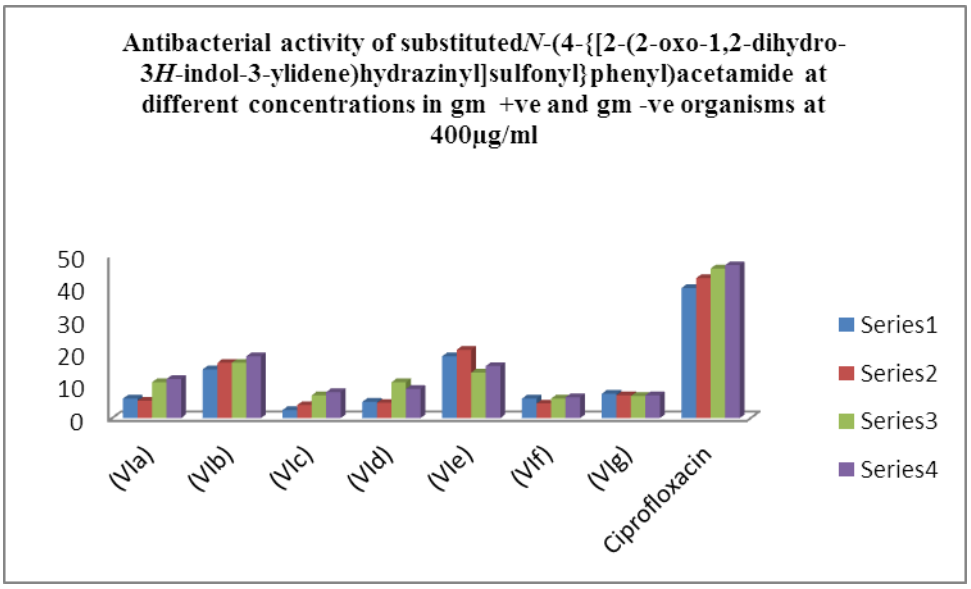


Table 3: Antifungal activity of substituted*N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazinyl]sulfonyl}phenyl)acetamide.

S.NO	Compound	C.albicans
1	(VIa)	12mm
2	(VIb)	18mm
3	(VIc)	9mm
4	(VId)	5mm
5	(VIe)	18.5mm
6	(VIf)	5.8mm
7	(VIg)	14mm
8	Nystatin	25mm

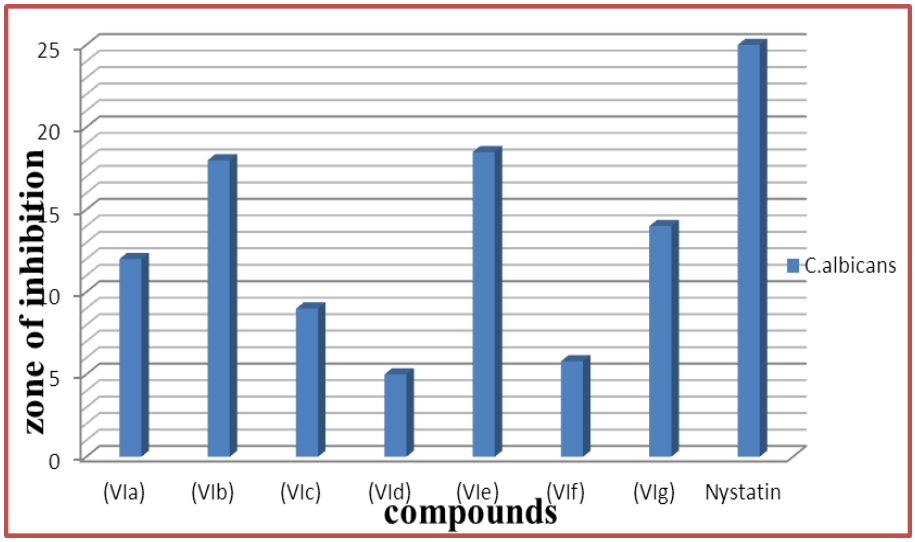
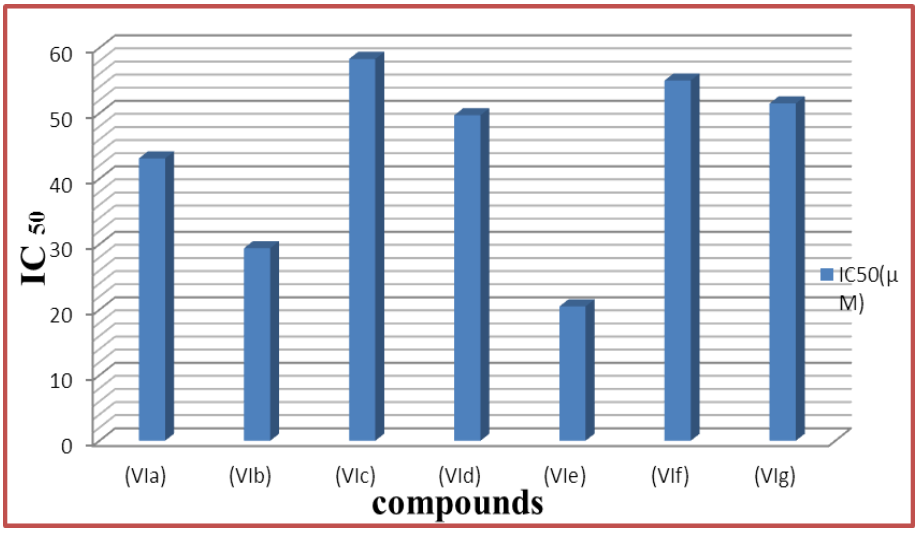


Figure 3: Graphical representation of substituted*N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazinyl]sulfonyl}phenyl)acetamide.

Antioxidant activity of substituted*N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) hydrazinyl]sulfonyl}phenyl)acetamide.

compound	IC_{50}(µM)
(VIa)	43.1
(VIb)	29.4
(VIc)	58.3
(VId)	49.7
(VIe)	20.5
(VIf)	55
(VIg)	51.5
Ascorbic acid	8.65

Graph showing antioxidant activity of*N*-(4-{[2-(2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)hydrazinyl]sulfonyl}phenyl)acetamide



ANTIMICROBIAL ACTIVITIES: The synthesized derivatives have been evaluated for their antimicrobial and antioxidant activities against Gram-positive (*S.aureus and B.subtilius*) and Gram-negative *(E.coli and P.aureginosa*) bacteria by measuring the zone of inhibition. The results have been compared with a broad spectrum antibacterial agent ciprofloxacin as standard drug. Among all the synthesized compounds, compound VIb (R=Cl), VIe(R=F) were found to be more effective antibacterial agents. The compounds VIf(R=COOH) showed minimum activity towards *S. aureus* at 400µg/ml with the zone of inhibition of 6mm.The compound VIc(R=NO_2) showed minimum activity towards *E.coli* at 400µg/ml concentration with the zone of inhibition of 2.5mm.The compound VId(R=CH_3) showed minimum activity towards at *P. aureginosa* 400µg/ml concentration with the zone of inhibition of 4.7mm.

ANTIFUNGAL ACTIVITY: The synthesized derivatives have been evaluated for their Antifungal activity. The results have been compared with a broad spectrum antifungal agent Nystatin as standard drug. Compounds VIe(R=F), VIb(R=Cl) showed maximum activity towards *C.albicans*. Compounds VIa(R=H), VIg(R=6-Br) showed moderate activity towards C.albicans. Compounds VIf(R= 7-COOH), VId(R=5-CH_3) showed poor activity towards *C.albicans*.

ANTIOXIDANT ACTIVITY: All the derivatives were having antioxidant activity. Amongst them, the compounds VIe(R=F) was found to be active than standard (ascorbic acid) and the compound VIc(R=NO_2) was found to be less active than standard (ascorbic acid). The compound VIe(R=F) was found to be more active than all the compounds tested. The compound VIc(R=NO_2) was found to be least active than all the compounds tested.

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