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Synthesis and Evaluation of New N-(2-Furan-3yl)-4-(2-Oxoindolin-3-Ylidene)-Thiazolidine-3yl)-2-Phenoxy Acetamides for Anti Microbial and Anti Oxidant Activity

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ABSTRACT

To synthesize the compounds by adopting appropriate synthetic routes. Purification and characterization of all the new compounds including those of intermediates by recrystallization or by using column chromatographic techniques. Characterization of newly synthesized compounds by physical and spectral methods. Evaluation of the new compounds for Anti-microbial activity as anti bacterial activity, anti fungal activity and Antioxidant activity. Among the series compound Vcwith(5-Br)showed potent antimicrobial activity with zone of inhibition 10mm,14mm,18mm and 14mm, 16mm, 18mmagainst *M.luteus, S. aureus*species and Vd(CH3) showed less antimicrobial aactivity with zone of inhibition 05mm, 10mm, 13mm and 05mm, 09mm, 12mmagainst *M.luteus, S. aureus*when compared with standard drug Amoxycillin. Among the series compounds Vcwith(5-Br) and Vg with(5-F) showed potent antifungal activity against *Candida albicans, Candida glabrata*species. Among all the compounds, compound Vc, Vh(R=5-F) showed good free radical scavenging activity with IC50 values of 20.23,21.43µM. Compounds Vb, Vj(R=5-Br,5-CH3) were found in the next order of free radical scavenging activity with IC50 values 30.43µM, 36.26µM respectively. None of the compounds showed anti-oxidant activity on a par with ascorbic acid with IC50 value 6.55µM.

KEYWORDS: Aromatic aldehyde, Acetic acid, Thioglycolic acid, Phenoxyacetyl hydrazide.

INTRODUCTION

Isatin or 1H-indole-2,3-dione is an indole derivative. The compound was first discovered by Erdmann and Laurent in 1841 as a product from the oxidation of indigo dye by nitric acid and chromic acids. Isatin is one of the important heterocyclic compounds. Recently, heterocyclic compounds, analogues and their derivatives have attracted strong interest in medicinal chemistry due to their biological and pharmacological properties. The small and simple isatin nucleus possesses numerous biological properties. **Thiazolidine** is a heterocyclic organic compound with the formula (CH2)3(NH)S. It is a 5 membered saturated ring with a thioether group and an amine group in the 1 and 3 positions. It is a sulfur analog of oxazolidine. Thiazolidine is a colorless liquid. Derivatives of **thiazolidines**, are known. For example, the drug pioglitazone contains a thiazolidine ring. Another drug that contains a thiazolidine ring is the antibiotic penicillin. Thiazolidine was used in the synthesis of homogeneous penicillamine disulphide cross-linked polypeptides. Used in the treatment of Diabetes Mellitus type-2. Penicillins consist of a thiazolidine ring connected to a β-lactam ring to which is attached by a side chain that determines many of the antibacterial and pharmacological characteristics of particular penicillins.

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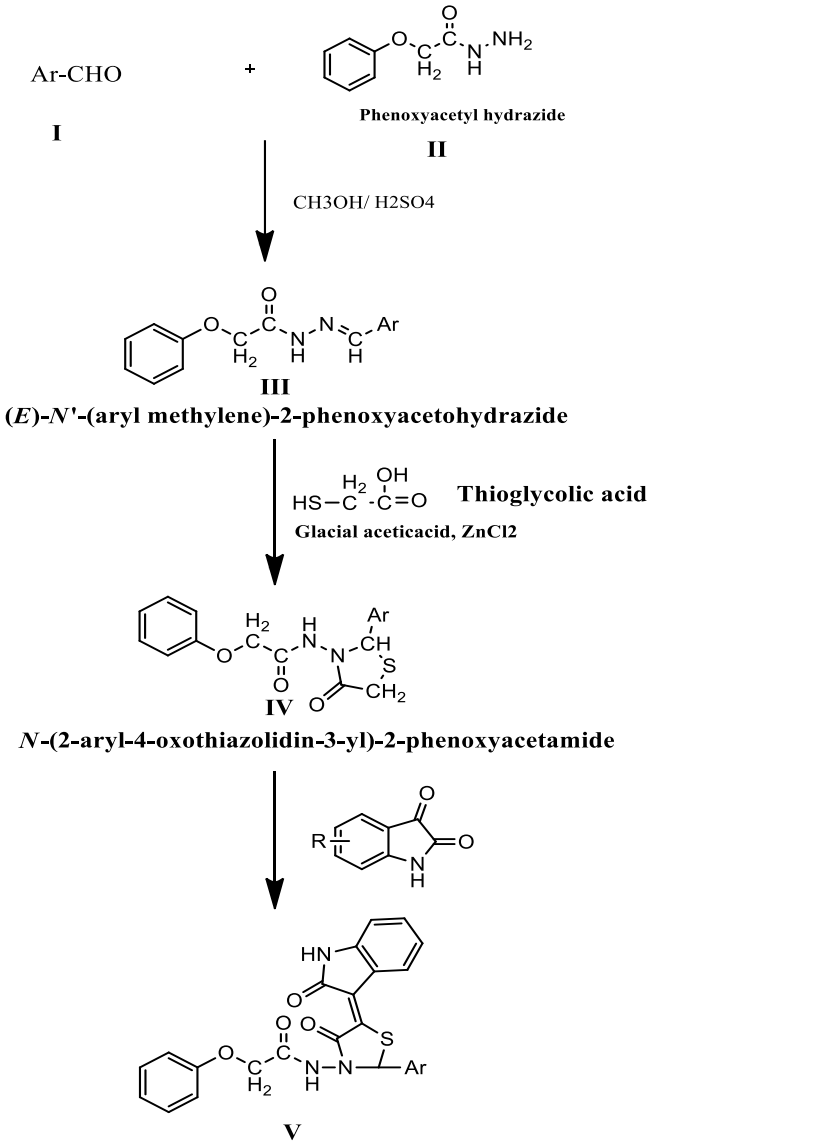
MARERIALS AND METHODS

Scheme:1



EXPERIMENTAL WORK:

Preparation of N-(4-oxo-5-(2-oxoindoline)-2-phenylthiazolidin-3yl)-2-phenoxyacetamide:

1)Preparation of N-benzylidine-2-phenoxyaceto hydrazide: To the Benzaldehyde (0.01Mol) (106.12g/mol)was taken in a beaker, to it add phenoxyacetyl hydrazine hydrate(I,0.01Mol)(166.18g/mol) drop by drop. Precipitate is formed during addition of phenoxyacetyl hydrazine, keep reflux for 10 hours by the addition of H2SO4& Methanol. Then to it add crushed ice& filter the product, the product was washed with ice cold water& dry it.

2) Conversion of N-(5-methyl-4-oxo-2-phenylthiazolidin-3yl)-2-phenoxyacetamide from N-benzylidine-2-phenoxyaceto hydrazide: To the above product (II,0.01Mol)(342.41g/mol), add thioglycollic acid (0.01Mol)(92.112g/mol) , 10ml of glacial aciticacid, CH30H and finally add a pinch of ZnCl2. The above mixture was reflux it for 10hours, cool it and poured into crushed ice and add NaCO3 solution. Filter the product. Washed it with cold water& dry it.

3)Preparation of N-(4-oxo-5-(2-oxoindolin-3-ylidene)-2-phenylthiazolidin-3-yl)-2-phenoxyacetamide: To the product (III,0.01Mol), add isatin and its derivatives(0.01Mol), glacial acetic acid 2-3 drops and methanol. Reflux the above mixture for 10 hours,cool it and poured into crushed ice. Precipitate was formed during the addition of ice. Filter and dry it.

ANTI BACTERIAL ACTIVITY OF SYNTHESISED COMPOUNDS:

Antibacterial activity by agar well diffusion method by measuring the zone of inhibition in mm.

MATERIALS:

Nutrient broth media, Nutrient agar media, Dimethyl sulfoxide, Ciprofloxacin, Distilled water

TEST ORGANISMS: Gram positive bacteria *Staphylococcus aureus* and *Micrococcus luteus*, Gram negative bacteria *Escherichia coli* and *Klebsiella pneumoniae.*

NUTRIENTMEDIA COMPOSITION:

Beef extract: 3grams, Peptone: 5 grams, Sodium chloride: 5 grams, Agar agar: 5grams

Distilled water:1000 litres, pH: 7.4±0.2

PREPARATION OF BACTERIAL CULTURES OF ASSAY:

The test organisms were sub cultured using nutrient broth medium. The tubes containing sterilized media were inoculated with respective bacterial strains. After incubation at37±1ºC for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculums were prepared by transferring a loop full of culture to nutrient broth in conical flasks. The flasks were inoculated at 37±1ºC for 48 hours before the experiment.

THE SAMPLE PREPARATION:

The test compounds were prepared for assay by dissolving them in dimethyl sulfoxide in required concentrations making 50µg/ml, 100µg/ml, 500µg/ml respectively for evaluation. A reference standard for both gram positive and gram negative bacteria ciprofloxacin was made in same concentrations as test compounds where dimethyl sulfoxide as control.

ASSAY BY AGAR WELL DIFUSSION METHOD:

The antibacterial susceptibility of test compounds was assessed by well diffusion method. The sterilized (autoclaved at 120ºC for 20min) about 25ml of nutrient agar medium was poured in sterile Petri dishes and plates were kept aside for solidification. Bacterial lawns were prepared by spreading the bacterial suspension (1ml/100ml i.e.,cfu/ml) on the surface of the agar plates by using sterile L-shaped glass rod. Wells were punched on the agar plates using sterile borer(6mm). The prepared concentrations (50µg/ml, 100µg/ml, and 300µg/ml) of test sample along with control were loaded into wells aseptically of about 50µl. The plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. Later the plates were incubated for 24 hours at 37±1ºC. After incubation period diameter of the zone of incubation of each well were measured manually by using a millimetre scale and tabulated. Whole experiment was carried out in triplicate. Simultaneously dimethyl sulfoxide was maintained as control to observe the solvent effect on bacteria.

ANTI FUNGAL ACTIVITY:

Anti fungal activity by agar well diffusion method by measuring the zone of inhibition in mm.

MATERIALS:

Nutrient broth media, Nutrient agar media, Dimethyl sulfoxide, Flucanazole, Distilled water

TEST ORGANISMS: Candida albicans, Candida

SABOURAUD DEXTROSE AGAR BROTH COMPOSITION:

Dextrose- 40g/L, Peptone-10g/L, Agar Agar-15g/L, Distilled water-1000Litres, PH: 7.4±0.2

PREPARATION OF FUNGI CULTURES FOR ASSAY:

The test organisms were sub cultured using SDA broth medium. The tubes containing sterilized media were inoculated with respective fungi strains. After incubation at 37±1ºC for 48hrs, they were stored in refrigerator. The stock cultures were maintained. The inoculums were prepared by transferring a loop full of culture to nutrient broth in conical flask. T he flasks were incubated at 37±1ºC for 48hrs before the experiment.

TEST SAMPLE PREPARATION:

The test compounds were prepared for assay by dissolving them in dimethyl sulfoxide in required concentrations making 50µg/ml, 100µg/ml, and 300µg/ml respectively for evaluation. A reference standard for both fungi Flucanozole was made in same concentrations as test compounds where dimethyl sulfoxide as control.

ASSAY BY AGAR WELL DIFFUSION METHOD:

The anti fungal susceptibility of test compounds was assessed by agar well diffusion method. The sterilized (autoclaved at 120ºC for 20mins) about 25ml of SDA medium was poured in sterile petri dishes and plates were kept aside for solidification. Fungal lawns were prepared by spreading the fungal suspension (1ml/100ml i.e., cfu/ml) on the surface of the agar plates by using sterile L-shaped glass rod. The wells were punched on the agar plates using sterile borer (6mm). The prepared concentrations 50µg/ml, 100µg/ml &300µg/ml) of test sample along with control were loaded into wells aseptically of about 50µl. The plates were kept undisturbed for at least 2hrs in refrigerator to allow diffusion of the solution properly into the SDA medium. Later the plates were incubated for 48hrs at 37±1ºC. After incubation period diameter of the zone of inhibition of each well were measured manually by using a mm scale & tabulated. Whole experiment was carried out in triplicate. Simultaneously dimethyl sulfoxide was maintained as control to observe the solvent effect on fungi.

ANTIOXIDANT ACTIVITY *(IN VITRO)*

Evaluation of anti-oxidant activity of new 1,3,4-Thiadiazole derivatives

Materials: Ascorbic acid :(Analytical grade, Merck India), Methanol :(HPLC grade, Merck India), DPPH :(α,α-diphenyl, β-picryl hydrazyl) (Sigma Aldrich), Test compounds, Double Distilled water.

Preparation of Standard Solution of Ascorbic Acid:

Required amount of Ascorbic acid was accurately weighed and dissolved in distilled water to prepare 1mM stock solution. Solutions of different concentrations (1nM, 3nM, 10nM, 30nM, 1µM, 3µM, 10µM, 30µM, 100µM, 300µM, 1mM) were prepared.

Preparation of DPPH Solution:

0.5mM solution of DPPH was prepared by dissolving the19.71mg of DPPH in 100ml of methanol. The solution was protected from sunlight to prevent the oxidation of DPPH.

Preparation of Test Compounds:

Required amount of test compounds was dissolved in methanol and 1mM stock solution was prepared. Solutions of concentration ranging from 1nM to 1mM were prepared.

Principle:

The method is based on principle described by Blois (1958) method. The model of scavenging the DPPH radical is most widely used method to evaluate the anti-oxidant activity in relatively shorter time compared with other methods. The effect of anti-oxidant on DPPH radical scavenging was thought to be their hydrogen donating ability (Baumann *et al., 2002*).

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517nm induced by anti-oxidants. The absorption maximum of stable DPPH radical in methanol was at 517nm. The decrease in absorbance of DPPH radical caused by anti-oxidants, because of the reaction between anti-oxidant molecules and radical, progresses, which result in the scavenging of the radical by hydrogen donation. Hence, DPPH is used as a substrate to evaluate the anti-oxidant activity.

Procedure:

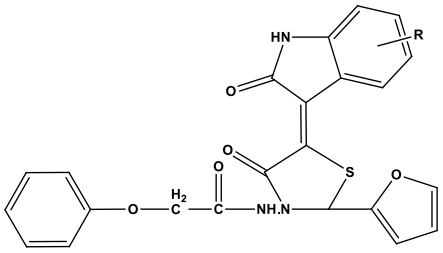
To 2.8ml of test sample/ascorbic acid, 0.2ml of DPPH solutionwas added, mixed thoroughly and absorbance was measured at 517nm against blank, prepared in an identical way but without the test compound. The results were plotted on a graph and IC50 value was calculated.

$$\%\text{Inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Test}}{\text{Absorbance of Blank}} \times 100$$

RESULTS AND DISCUSSION

Synthetic work of these studies has positively undergone as per the planning and as such in all the reactions carried, the expected compounds alone could be obtained. All the above mentioned new bis isatin derivatives were characterized by Physical and spectral data.

Physical data ofN-(2-Furan-3yl)-4-(2-Oxoindolin-3-ylidene)thiazolidine-3yl)-2-phenoxy acetamide derivatives (Va-Vf):



N-(2-Furan-3yl)-4-(2-oxoindolin-3-ylidene)thiazolidine-3yl)-2-phenoxy acetamides

Table No: 1

S.NO	Compound	R	Molecular Formula	Molecular Weight	Melting Point (°C)	Yield
1	V-a	H	$C_{23}H_{17}N_3O_5S$	447.46	154-157	61%
2	V-b	5-Br	$C_{23}H_{16}BrN_3O_5S$	526.36	172-175	45%
3	V-c	7-Cl	$C_{23}H_{16}ClN_3O_5S$	481.91	202-204	79%
4	V-d	5-F	$C_{23}H_{16}FN_3O_5S$	465.45	175-177	58%
5	V-e	5-CH3	$C_{24}H_{19}N_3O_5S$	461.49	206-208	80%

SPECTRAL DATA:

IR spectrum (KBr, cm^{-1}):3444.03 (N-H amide) 3058.52–(C-H Aromatic (str)), 2975.75–(C-H aliphatic (str)), 1692.62–(C=O (str)), 1537.07–(C=C Aromatic(str)), 1219.06-(C-O(str));

^{1}H NMR (300 MHz) (DMSO):11.25(s, 1H, indole, NH), 8.94-8.96 (d, 1H, Ar-H), 7.60 (S, NH, amide), 7.57-7.59 (t, 2H, Ar-H), 7.26-7.41 (m, 4H, Ar-H), 7.15-7.17(d, 1H, Ar-H), 6.96-7.00(m, 3H, Ar-H) 6.32-6.36 (m,2H, furanyl), 6.15 (s,1H, thiazole) 4.96(s, 24H, ethoxy).

Mass spectrum (ESI) M+1 peak was observed at 448.24.

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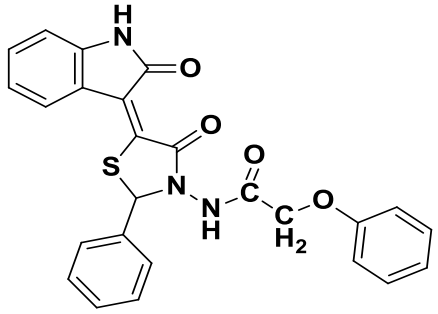

Physical data of N-(4-Oxo-5-(2-oxoindolin-3-ylidene)-2-phenyl-thiazolidin-3-yl)-2-phenoxyacetamide (Vf- Vj).

Table No: 2

S.NO	Compound	R	Molecular Formula	Molecular Weight	Melting Point(°C)	Yield
1	V-f	H	$C_{25}H_{19}N_3O_4S$	457.50	148-150	43%
2	V-g	5-Br	$C_{25}H_{18}BrN_3O_4S$	536.40	156-159	59%
3	V-h	7-Cl	$C_{25}H_{18}ClN_3O_4S$	491.95	194-197	36%
4	V-i	5-F	$C_{25}H_{18}FN_3O_4S$	475.49	178-180	28%
5	V-j	5-CH3	$C_{26}H_{21}N_3O_4S$	471.53	125-130	40%

IR (KBr, cm^{-1}): 3428.45(NH), 2924.87(CH), 1681.39(C=O), 1645.14(C=N).

^{1}H NMR (300 MHz) (DMSO) :11.22 (s, 1H, indole, NH), 8.94-8.96 (d, 1H, Ar-H) 8.09-8.11(d, 2H, Ar-H), 7.60 (S, NH, amide), 7.55-7.59 (t, 1H, Ar-H), 7.49-7.51 (m, 3H, Ar-H), 7.27-7.34(m, mH, Ar-H), 7.15-7.17(d, 1H, Ar-H), 6.96-7.00(m, 3H, Ar-H) 5.92 (s,1H, thiazole), 4.96(s, 2H, ethoxy).

Mass spectrum (ESI) M+1 peak was observed at 458.17.

ANTI BACTERIAL ACTIVITY OF SYNTHESISED COMPOUNDS:

Table No: 3 Anti bacterial activity on *Kebsilla pneumonia*

Ar	Compound	R	50 µg/ml (mm)	100µg/ml (mm)	300µg/ml (mm)
furfuryl	Va	H	06	12	15
	Vb	7-Cl	07	10	14
	Vc	**5-Br**	**10**	**14**	**18**
	Vd	5-CH3	05	10	13
	Ve	5-F	07	10	14
Phenyl	Vf	H	06	12	14
	Vg	7-Cl	05	09	12
	Vh	**5-Br**	**08**	**12**	**16**
	Vi	5-CH3	05	10	14
	Vj	5-F	04	08	12
	Amoxycillin	-	12	16	22

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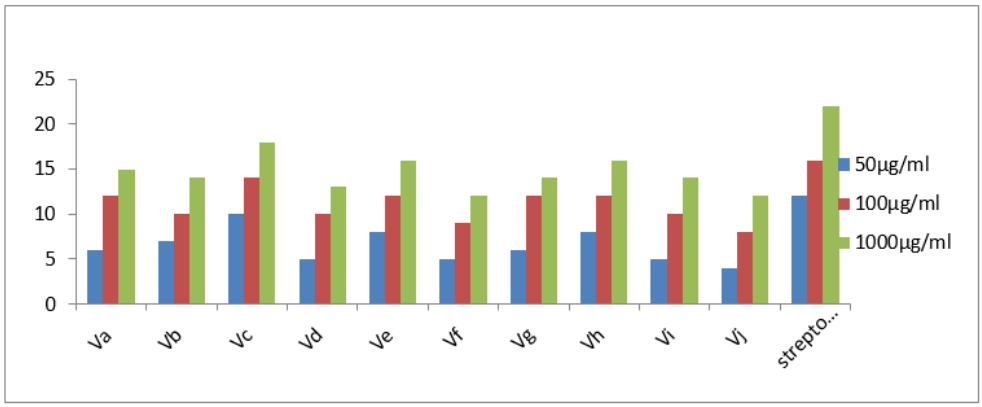

Fig No: 1 Graphical data of *Kebsilla pneumonia*

Table No: 4 Antibacterial activity on *Micrococcus luteus*

Ar	Compound	R	50 µg/ml (mm)	100µg/ml (mm)	300µg/ml (mm)
furfuryl	Va	H	08	10	16
	Vb	7-Cl	06	12	14
	Vc	**5-Br**	**12**	**16**	**18**
	Vd	5-CH3	05	09	12
	Ve	5-F	08	10	14
Phenyl	Vf	H	06	06	11
	Vg	7-Cl	**08**	**12**	**15**
	Vh	**5-Br**	05	09	12
	Vi	5-CH3	08	12	14
	Vj	5-F	07	10	12
	Amoxycillin	-	14	18	24

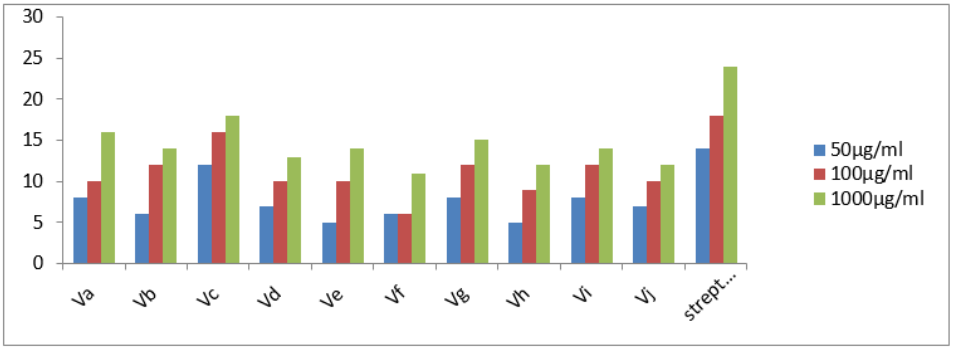

Fig No: 2 Graphical data of *Micrococcus luteus*

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Table No: 5 Antibacterial activity on Staphylococcus aureus

Ar	Compound	R	50 µg/ml (mm)	100µg/ml (mm)	300µg/ml (mm)
furfuryl	Va	H	10	12	15
	Vb	7-Cl	07	10	14
	Vc	**5-Br**	**12**	**16**	**18**
	Vd	5-CH3	05	10	13
	Ve	5-F	07	12	14
Phenyl	Vf	H	**05**	**08**	**12**
	Vg	7-Cl	06	12	14
	Vh	**5-Br**	07	10	12
	Vi	5-CH3	05	08	10
	Vj	5-F	08	10	14
	Amoxycillin	-	12	16	22

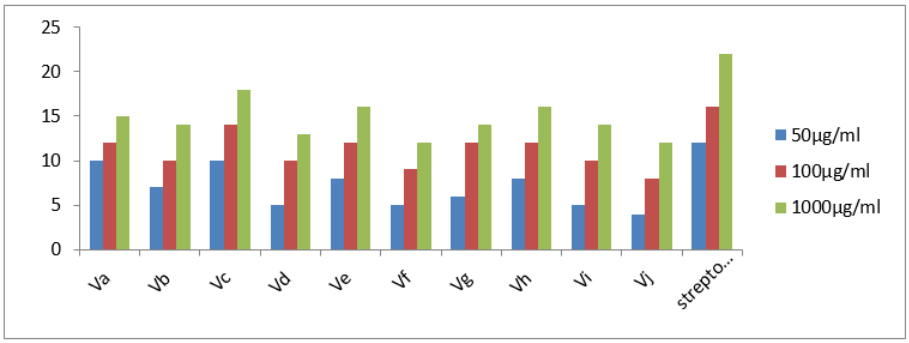

Fig No: 3 Graphical data of Staphylococcus aureus

Table No: 6 Antibacterial activity on Escherichia coli

Ar	Compound	R	50 µg/ml(mm)	100µg/ml(mm)	300µg/ml(mm)
furfuryl	Va	H	08	10	14
	Vb	7-Cl	07	10	12
	Vc	**5-Br**	**12**	**16**	**20**
	Vd	5-CH3	05	12	12
	Ve	5-F	06	10	14
Phenyl	Vf	H	**05**	**09**	**12**
	Vg	7-Cl	07	12	14
	Vh	**5-Br**	08	12	16
	Vi	5-CH3	05	10	14
	Vj	5-F	04	08	12
	Amoxycillin	-	14	18	22

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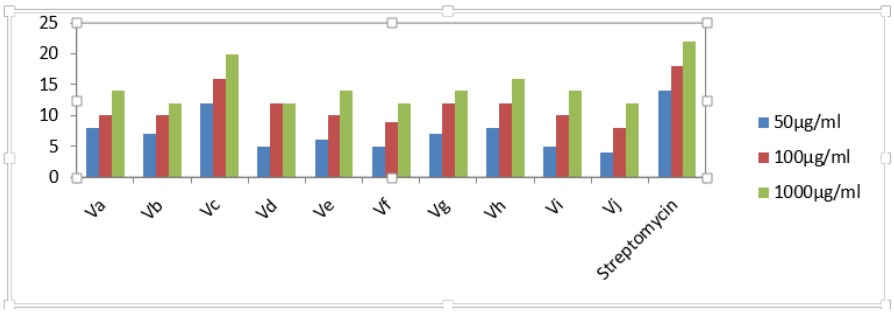

Fig No: 4 Graphical data of Escherichia coli

Among the series compound **Vc**with**(5-Br)**showed potent antimicrobial activity with zone of inhibition **10mm,14mm,18mm** and **14mm, 16mm, 18mm**against ***M.luteus, S. aureus***species and **Vd(CH3)** showed less antimicrobial aactivity with zone of inhibition 05mm, 10mm, 13mm and 05mm, 09mm, 12mmagainst ***M.luteus, S. aureus***when compared with standard drug **Amoxycillin.** Among the series compound **Vc and Vf**with **(5-Br)** showed potent antimicrobial activity with zone of inhibition **12mm, 16mm, 18mm** and **12mm, 16mm, 20mm** against ***Staphylococcus aureus, Escherichia coli*** when compared with standard drug **Amoxycillin.** Furfuraldehyde containing moieties shows more potent antimicrobial and antifungal anti oxidant activity compared with phenyl containing moieties.

ANTI FUNGAL ACTIVITY:

Anti fungal activity by agar well diffusion method by measuring the zone of inhibition in mm.

Table No: 7 Antifungal activity on *Candida albicans*

Ar	Compound	R	50 µg/ml (mm)	100µg/ml (mm)	300µg/ml (mm)
furfuryl	Va	H	06	10	12
	Vb	7-Cl	03	05	08
	Vc	**5-Br**	**08**	**12**	**14**
	Vd	5-CH3	07	10	12
	Ve	5-F	05	08	10
Phenyl	Vf	H	06	08	11
	Vg	7-Cl	05	08	10
	Vh	**5-Br**	04	06	09
	Vi	5-CH3	05	08	10
	Vj	5-F	06	09	12
	Ketoconozole	-	12	16	18

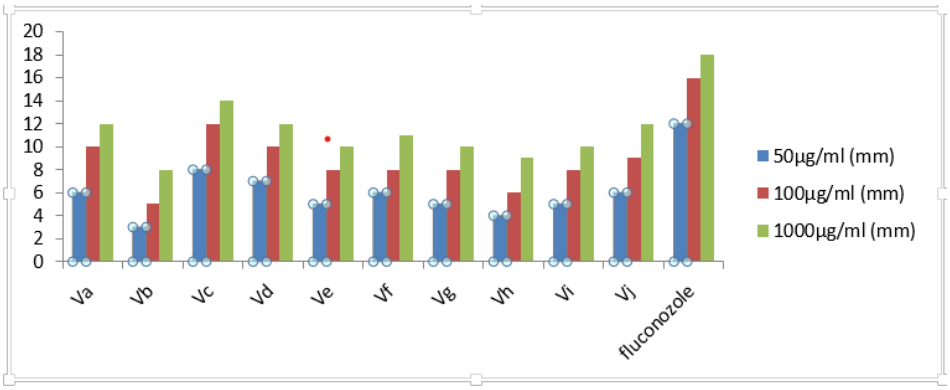

Fig No: 5 Graphical data of *Candida albicans*

Table No: 8 Antifungal activity on *Candidaglobrata*

Ar	Compound	R	50 µg/ml (mm)	100µg/ml (mm)	300µg/ml (mm)
furfuryl	Va	H	**06**	**08**	**10**
	Vb	7-Cl	04	06	09
	Vc	**5-Br**	**08**	**10**	**14**
	Vd	5-CH3	05	07	09
	Ve	5-F	06	08	12
Phenyl	Vf	H	04	06	08
	Vg	7-Cl	05	08	10
	Vh	**5-Br**	**04**	**06**	**09**
	Vi	5-CH3	05	08	10
	Vj	5-F	04	07	09
	Ketoconozole	-	10	14	18

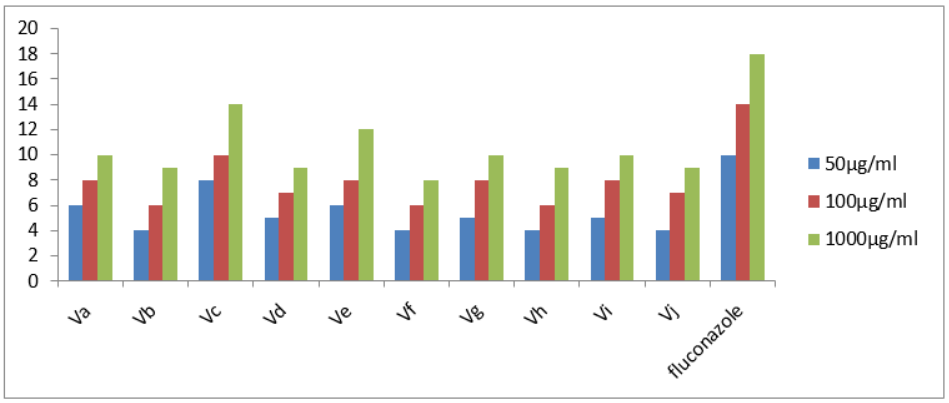

Fig No:6 Graphical data of *Candidaglobrata*

Among the series compounds **Vc** with **(5-Br) and Vg** with **(5-F)** showed potent antifungal activity against *Candida albicans*, *Candida glabrata* species.

ANTIOXIDANT ACTIVITY *(IN VITRO)*

Table No: 9 anti-oxidant activity of new 1,3,4-Thiadiazole derivatives

Ar	Compound	R	**IC50(µM)**
furfuryl	Va	H	54.21
	Vb	7-Cl	30.43
	Vc	**5-Br**	20.25
	Vd	5-CH3	42.56
	Ve	5-F	39.32
Phenyl	Vf	H	60.12
	Vg	7-Cl	56.23
	Vh	**5-Br**	21.40
	Vi	5-CH3	36.26
	Vj	5-F	46.23
	Ascorbic acid	-	6.5

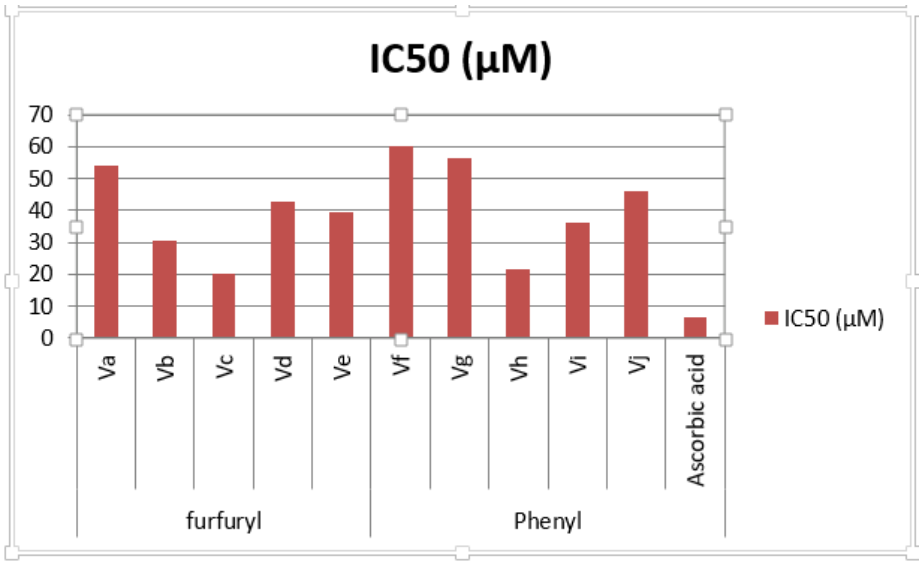

Fig No: 7 Graphical data of anti oxidant activity

Among all the compounds, compound Vc, Vh(R=5-F) showed good free radical scavenging activity with IC50 values of 20.23,21.43µM. Compounds Vb, Vj(R=5-Br,5-CH3) were found in the next order of free radical scavenging activity with IC50 values 30.43µM, 36.26µM respectively. None of the compounds showed anti-oxidant activity on a par with ascorbic acid with IC50 value 6.55µM.

CONCLUSION

The synthesized compounds are purified by column chromatography. Compounds are characterized by the analytical and spectral (IR, ^{1}H NMR and Mass) data. The synthesized compounds were evaluated for anti bacterial activity, anti fungal activity and Antioxidant activity. Among the series compound Vc with(5-Br)showed potent antimicrobial activity with zone of inhibition 10mm,14mm,18mm and 14mm, 16mm, 18mmagainst *M.luteus, S. aureus*species and Vd(CH3) showed less antimicrobial activity with zone of inhibition 05mm, 10mm, 13mm and 05mm, 09mm, 12mmagainst *M.luteus, S. aureus* when compared with standard drug Amoxycillin. Among the series compound Vc and Vfwith (5-Br) showed potent antimicrobial activity with zone of inhibition 12mm, 16mm, 18mm and 12mm, 16mm, 20mm against *Staphylococcus aureus, Escherichia coli* when compared with standard drug Amoxycillin. Furfuraldehyde containing moieties shows more potent antimicrobial and antifungal anti oxidant activity compared with phenyl containing moieties.

Among the series compounds Vc with (5-Br) and Vg with(5-F) showed potent antifungal activity against *Candida albicans, Candida glabrata* species. Among all the compounds, compound Vc, Vh (R=5-F) showed good free radical scavenging activity with IC50 values of 20.23,21.43µM. Compounds Vb, Vj (R=5-Br,5-CH3) were found in the next order of free radical scavenging activity with IC50 values 30.43µM, 36.26µM respectively. None of the compounds showed anti-oxidant activity on a par with ascorbic acid with IC50 value 6.55µM.

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