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In-Vitro Evaluation of some Novel 5, 5-disubstituted -1, 2, 4-triazolidine-3-one derivatives

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Abstract

Conventional synthesis of 5, 5-Disubstituted -1, 2, 4-Triazolidine-3-One derivatives have been synthesized at laboratory scale by condensation of oxazol-5-ones by reaction of hippuric acid and different aldehydes with thiosemicarbazide. The compounds structures were characterized by IR, H^1 NMR and C^{13} NMR. All the compounds have been evaluated for *invitro* antimicrobial and antioxidant activity and were compared with their corresponding standards. Of all the synthesized derivatives compounds 1a,10a,12a exhibited good antimicrobial properties.

Key-Words: 1, 2, 4 triazole 3-one, antioxidant, antifungal, antibacterial, heterocycle

Introduction

Triazole is a five-membered heterocyclic ring constituting two carbon atoms and three nitrogen atoms. Triazole exists in three isomeric forms of which 1, 2, 4-triazole is of pharma ceutical importance. The positions of nitrogen atoms present in the ring were responsible for the binding of enzymes and receptors in biological system through non-covalent interactions and exhibit significant biological activity. From literature survey 1, 2, 4 triazoles possess low toxicity and good pharmacokinetic and pharmacodynamic profiles exhibiting biological activities such as antibacterial [1], antifungal [2], antiviral [3], anticonvulsant [4], anti-inflammatory [5], analgesic [6], antitumor [7], antitubercular [8], anti-convulsant [9], anticancer [10], antimalarial [8], antimigraine [8], potassium channel activators[8], hypoglycaemic [11-12], antidepressant [13], anti-proliferative [14],and antioxidant [15].There are several methods for the synthesis of 1,2 ,4-triazoles from diacylhydrazide, acyl thiocyanates, esters, thiosemicarbazone. From amino guanidine and formic acid,[16] amidines and nitriles [17], thioamides and hydrazides [18], acyl hydrazines[19], semicarbazones with ferric chloride[20] , substituted hydrazine and formamide, 1,3,4-oxadiazol-5-thiones.[21-23]

Due to the high significance of 1,2,4 triazoles the present work was focussed on the development of novel synthetic method and invitro evaluation of 1,2,4 triazole derivatives that is 5,5-disubstituted 1,2,4-triazolidine-3-one derivatives by condensation of oxazol-5-ones with semicarbazide.

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Material and Methods

Melting points were determined in open glass capillaries using GallenKamp (MFB-600) melting point apparatus and were uncorrected. IR spectra (KBr discs) Bruker analyzers were confirmed by Shimadzu FT-IR Spectrophotometer using KBr pellets technique, Model No.8400S (Japan). 1H NMR spectra were recorded on Bruker 300 MHz NMR spectrometer (Switzerland) using DMSO as solvent. T.L.C. was run on silica gel G plates using ethyl acetate: n-hexane (7:3) as developing solvent for the purity of the compounds. All other chemicals used in the present studies were either of A.R or G.R quality.

Drugs and chemicals

Hippuric acid – (LOBA-B.NO-G228507), Acetic anhydride – (FISHER'S SCIENTIFIC-B.NO-92757004-2),Sodium Acetate – (FINAR-B.NO-19095780),semicarbazide –(LOBA-B.NO-S057310) ,Methanol (SD FINE-CHEMLIMITED- B.NO-IOZA-0502-0409-13),Charcoal – (QUALINGENS-BNO-17335406-S) ,Ethanol – (CSS-B.NO-110605), N,N-Dimethylformamide (DMF) – (LOBAB.NO-LIO1571306).

General procedure for the Synthesis of 5, 5-disubstituted -1, 2, 4-triazolidine-3-one derivatives (1a-12a)

The synthesis was carried out in two steps: **step-1** synthesis of 4-benzylidene-2-phenyloxazol-5(4H)-ones (1-12) by condensation of 0.01 moles of hippuric acid with 0.02 moles of different types of aromatic aldehydes in presence of 0.075 moles of acetic anhydride and 0.025 moles of sodium acetate with 2ml of water refluxed for 3hr. The reaction mixture was

cooled; precipitate was filtered, dried, recrystallized from methanol. **Step-2**: **Synthesis of 5, 5-disubstituted -1, 2, 4-triazolidine-3-one derivatives (1a-12a)**

The product from step1 condensed with equimoles (0.001 moles) of semicarbazide in presence of methanol and a few drops of 40% KOH refluxed for 6hrs, cooled; the product formed was filtered, dried and recrystallized from methanol. The progress and the purity of the reaction were confirmed by thin layer chromatography and melting point. The procedure was illustrated under **Scheme 1** and the physical data were tabulated in **Table 1**.the structural characterisation of synthesised compounds was done and were tabulated in **Tables 2, 3 and 4**

Ferric reducing antioxidant power (FRAP Assay) [24]

In ferric reducing antioxidant power assay,1ml of test sample of DMF(N,N-Dimethyl formamide) extract in different concentrations were mixed with 1ml of 0.2M sodium phosphate buffer (p^H-6.6) and 1ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in a temperature-controlled water bath at 50^0C for 20 min followed by addition of 1ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernant obtained (1ml) was added with 1ml of deionised water 200µl of 0.1%$FeCl_3$. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A_{700} after blank subtractions .the results were represented in **Table.5**

Antibacterial activity [25]

All the synthesized compounds 1a-12a were examined for *invitro* antibacterial activity against an assortment of two gram-positive bacteria *Staphylococcus aureusNCIM2901, Bacillus subtilis MTCC 441* and two Gram-negative bacteria *Pseudomonas aeruginosa* and *Proteus vulgaris MTCC 1771* by diffusion method. Tetracycline and Chloramphenicol were used as an internal standard.

Nutrient agar (High media) was dissolved and distributed in 25ml quantities in boiling tubes and were sterilized in an autoclave at 121^0C (15 lbs / sq.in) for 20 minutes. The medium was inoculated at one percent level using 18 hrs old cultures of the test organism mentioned above aseptically into sterile petridishes and allowed to set at room temperature for above 30min. In a size of 4 inches petridishes, five cups of 8mm diameter at equal distance were made in each plate. In the cups the test solutions of different concentrations were added and in another plate cups were made for standard and control. The plates thus prepared were left for 90 minutes in a refrigerator for diffusion. After incubation for 24 hours at 37^0C the plates were examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition measured and recorded. The results were represented in **Table 6**.

Antifungal activity [26]

The antifungal activity of compounds was assayed against three different fungal strains *Aspergillus niger MTCC 282, Penicillium chrysogenum MTCC 5108* and *Penicillium notatum NCIM 742.*

Potato dextrose agar (Hi- media) was dissolved and distributed in 25 ml quantities in 100ml conical flasks and were sterilized in an autoclave at 121 °C (15lbs/sq.in) for 20 minutes. The medium was inoculated with 1% 18hr old cultures of organisms aseptically in to sterile petridish and allowed to set at room temperature for about 30 minutes. At a size of 4 inches petridish 5 cups of 8mm diameter at equal distance were made in a petriplate with a sterile borer. The solutions of test concentrations (250µg/ml, 200µg/ml, 150µg/ml and 100µg/ml) and standard were added to respective cups aseptically and labelled accordingly. DMF as control did not show any inhibition. The plates were left for 90 minutes in refrigerator for diffusion and incubated for 72 hrs at $37^0 \pm 1^0$c. The plates were examined for inhibition zones. Fluconazole was used as standard. The experiments were performed in duplicate and the average diameters of the zones of inhibitions were summarized in **Table 7**

Results and Discussion

The starting compounds 4-benzylidene-2-phenyloxazol-5(4H)-one derivatives (1-12) react with corresponding semicarbazide in presence of potassium methoxide refluxed for 6 hrs to give **5, 5-disubstituted -1, 2, 4-triazolidine-3-one derivatives (1a-12a)** respectively. The structures of the compounds were established through IR, 1H and 13C NMR spectral data. The IR spectra of (1a-12a) exhibited absorption bands for secondary amine (-NH) at 3450-3350, cm-1, (-N-N) at 1240-1280 cm-1 , imines (–C=N–) at 1540-1501 cm-1, imines (–C=O–) at 1720-1690 cm-1, alkenes (–C=C–) 1640-1599 cm-1 . The 1H NMR spectra of these compounds revealed signals at δ = 7.65-7.98 ppm a multiplet for aromatic protons , δ = 2.0 ppm a doublet for(C-NH), δ = 8.11 ppm a singlet for(N=CH). The 13C NMR spectra of these compounds revealed signals at δ = 120-130 ppm peaks for aromatic carbons , δ = 130-134 ppm for(C-N), δ = 160.9 ppm for(N=C),), δ = 123.3,129.5

ppm for alkene carbons (–C=C-), δ = 160.14ppm for carbonyl carbon (-C=O).

All the compounds (1a-12a) were tested for antibacterial activity against an assortment of two gram-positive bacteria *Staphylococcus aureus NCIM 2901, Bacillus subtilis MTCC 441* and two gram-negative bacteria *Pseudomonas aeruginosa ,Proteus vulgaris MTCC 1771.* Tetracycline and Chloramphenicol were used as standards. For antifungal activity against three fungal strains *Aspergillus niger MTCC 282, Penicillium chrysogenum MTCC 5108* and *Penicillium notatum NCIM 742.* Fluconazole was used as standard.

The results of antimicrobial activities of synthesized compounds were shown in **Table 6 and 7** .All the compounds showed significant antibacterial and antifungal activity but more active towards gram positive bacteria .of all the derivatives synthesized compounds **1a, 10a, 12a** exhibited good and compounds **2a, 6a, 7a, 8a, 9a** showed moderate antimicrobial properties .

The structure-activity relationship studies based on the above *in vitro* results clearly indicate that compounds with moderate electron donating groups mainly mono and dialkyl substituted amino group, methoxy on the aromatic ring showed increased potency when compared to the strong electron donating groups such as hydroxy. the presence of halo groups mainly chlorine also showed good activity. The intense activity of the compounds is also greatly influenced by the position of the groups on the ring. The para substitution showed higher significant activity when compared to the electron donating groups at ortho position which clearly indicates that para substitution is responsible for increased activity. The results also indicate the influence of rise in activity with increase in the number of alkyl and alkoxy groups mainly methyl or methoxy substituent.

Conclusion

A series of new **5, 5-disubstituted -1, 2, 4-triazolidine-3-one derivatives (1a-12a)** were prepared by conventional method and evaluated for their *In-vitro* antimicrobial, ferric oxide reducing properties for which the mechanisms underlying this process remain to be fully elucidated. It is intended that the results from these studies will assist in elucidating their precise mechanism of action and provide an approach for further optimization and development to get new leads in the treatment of microbial infections.

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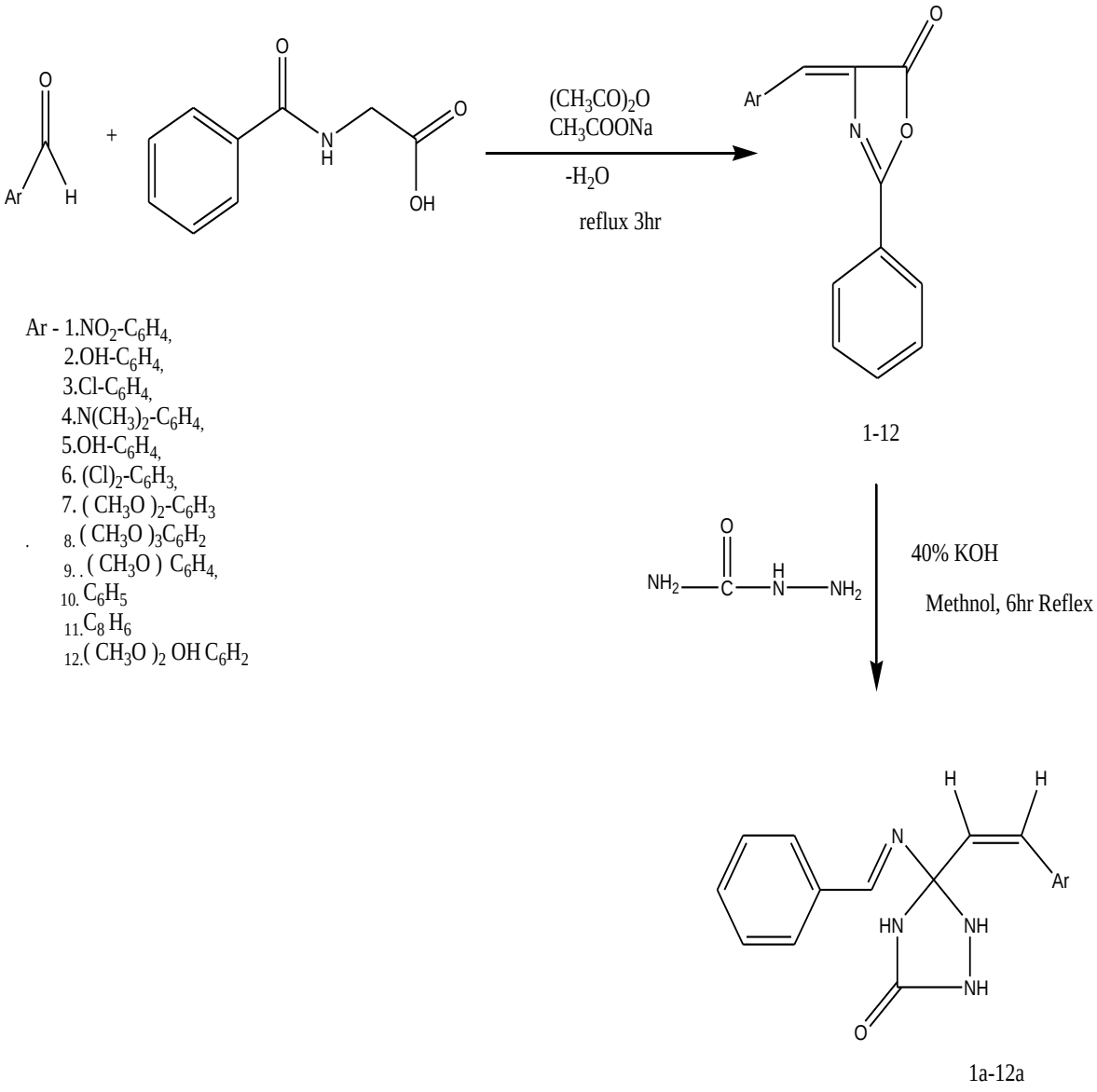

Ar - 1.NO_2-C_6H_4,
2.OH-C_6H_4,
3.Cl-C_6H_4,
4.$N(CH_3)_2$-C_6H_4,
5.OH-C_6H_4,
6. $(Cl)_2$-C_6H_3,
7. $(CH_3O)_2$-C_6H_3
8. $(CH_3O)_3C_6H_2$
9. . (CH_3O) C_6H_4,
10. C_6H_5
11. C_8H_6
12. $(CH_3O)_2$ OH C_6H_2

1a-12a

Scheme 1

Table 1: Physical Data

Code	Compound	M.F	M.W	MP(^{0}C)	%Yield	C%	H%	O%	N%	Cl%
1a	(5E)-5-(htdroxystyryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{17}H_{16}N_4O_2$	308	243^{0}C	63%	66.22	5.23	10.38	18.17	_
2a	(5E)-5-(4-nitrostyryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{17}H_{15}N_5O_3$	337	228^{0}C	64%	60.53	4.48	14.23	20.76	_
3a	5E)-5-(4-methoxystyryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{18}H_{18}N_4O_2$	322	204^{0}C	62%	67.07	5.63	9.93	17.38	_
4a	(5E)-5-(2,3-dichlorostyryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{17}H_{14}Cl_2N_4O$	360	230^{0}C	60%	56.52	3.91	4.43	15.51	19.63
5a	(5E)-5-(4-chlorosryryl)5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{17}H_{15}ClN_40$	326	231^{0}C	61%	62.48	4.63	4.90	17.15	10.85
6a	(5E)-5-(Z)-2-(1H-indol-3-yl)vinyl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{19}H_{17}N_5O$	331	229^{0}C	62%	68.87	5.17	4.83	21.13	_
7a	(5E)-5-(4-hydroxy-3,5-dimethoxystyryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{19}H_{20}N_4O_4$	368	232^{0}C	64%	61.95	5.47	17.37	15.21	_
8a	(5E)-5-(2,4-dimethoxystyryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{19}H_{20}N_4O_3$	352	211^{0}C	66%	64.76	5.72	13.62	15.90	_
9a	(5E)-5-(4-hydroxystyryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{17}H_{16}N_4O_2$	308	244^{0}C	65%	66.22	5.23	10.38	18.17	_
10a	(5E)-5-(3,4,5-trimethoxystyryl)-5-(benzylideneamino)-1,2,4-triazole-3-one	$C_{20}H_{22}N_4O_4$	382	216^{0}C	63%	62.82	5.80	16.74	14.65	_
11a	(5E)-5-(benzylideneamino)-5-styryl-1,2,4-triazole-3-one	$C_{17}H_{16}N_4O$	292	216^{0}C	67%	69.85	5.52	5.47	19.17	_
12a	(5E)-5-(4-(dimethylamino)styryl)-5-(benzylideneamino)-1,2,4-triazolidine-3-one	$C_{19}H_{21}N_50$	335	212^{0}C	65%	68.04	6.31	4.77	20.88	_

Table 2: IR Values

Compound	C-H	C=C	-NH-2º	C=N	O-H	Ar-NO2	C-Cl	C=O	O-CH_3
1a	3006	1600	3393	1529	3288			1702	
2a	2887	1642	3421	1518		1518		1718	
3a	3087	1640	3449	1520				1698	1244
4a	2918	1617	3362	1517			779	1722	
5a	3012	1614	3434	1541			641	1701	
6a	3032	1651	3412	1541				1711	
7a	3008	1657	3417	1542	3239			1709	1298
8a	3082	1648	3529	1512				1712	1236
9a	2988	1636	3429	1536	3208			1709	
10a	2932	1623	3377	1541				1697	1112
11a	2919	1650	3397	1550				1698	
12a	3085	1642	3390	1543				1708	

Table 3: 13C NMR

Compound	Conjugated vinylic Carbons (-C=C)	Aromatic carbons (Ar-C)	Ar-OH	C=N	O-CH_3	Ar- NO2	-C=O	C-Cl
1a	125.05,124.16	134.79,133.44,132.26,130.89,130.25,128.64,128.30,128.13,127.59,127.06,127.02	157.81	161.17			171.31	
2a	123.3,129.5	129.2,128.9,131.1,128.9,129.2,139.9,141.3,127.3,123.8,123.8,127.3		160.9		147.2	159.8	
3a	123.3,129.5	129.2,128.9,131.1,128.9,129.2,139.9,127.4,114.2,114.2,127.4,127.5		160.9	55.9		159.8	
4a	159.57	133.89,133.52,131.37,131.08,127.82,127.77		166.37			174.28	
5a	123.3,129.5	139.9,129.2,128.9,131.1,128.9,129.2,127.8,128.8,127.8,133.3		160.9			159.8	133.5
6a	129.3,129.5	129.2,128.9,131.1,128.9,129.2,139.9,126.1,119.0,122.2,120.1,111.1,135.5,130.8,110.1		160.9			159.8	
7a	129.3,129.5	129.2,128.9,131.1,128.9,129.2,139.9,126.1,119.0,122.2,120.1,111.1,135.5,130.8,110.1	132.0	160.9	56.2		159.8	
8a	129.3,129.5	129.2,128.9,131.1,128.9,129.2,139.9,100.3,106.5,		160.9	56.3,55.9		159.8	

		128.4,107.3						
9a	129.3,129.5	129.2,128.9,131.1,128.9, 129.2,139.9,127.8,115.8, 157.7,115.8,127.8,127.8	157.7	160.9	56.3,55.9		159.8	
10a	104.16,103.92	139.18,139.06,138.34,13 0.34		156.78	56.04,55.91 ,55.68		153.78	
11a	123.3,129.5	139.9,129.2,128.9,131.1, 128.9,129.2,135.2,126.4, 128.7,128.0,128.7126.4		160.9			159.8	
12a	123.3,129.5	129.2,128.9,131.1,128.9, 129.2,139.9,124.7,127.3, 114.2,127.3		160.9			159.8	

Table 4: 1HNMR

Compound	Hydrogen(n)	δ (ppm)	Multiplicity	Solvent
1a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.89,5.94 7.375-7.991 7.51,7.50	Singlet Multiplet doublet	DMSO
2a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	6.06,6.69 7.29-8.14 6.0	Singlet Multiplet doublet	DMSO
3a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.76,6.55 6.72-7.62 6.0	Singlet Multiplet doublet	DMSO
4a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.902 7.423-7.596 7.225	Singlet Multiplet doublet	DMSO
5a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	6.5.76,6.55 7.22-7.62 6.0	Singlet Multiplet doublet	DMSO
6a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.76,6.55 7.0-7.62 6.0	Singlet Multiplet doublet	DMSO
7a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.76,6.55 6.20-7.62 6.0	Singlet Multiplet doublet	DMSO
8a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.92,6.82 6.23-7.62 6.0	doublet Multiplet doublet	DMSO
9a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.76,6.55 6.68-7.62 6.0	Singlet Multiplet doublet	DMSO
10a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.76,6.55 7.756-7.019 6.549	Singlet Multiplet doublet	DMSO
11a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.76,6.55 7.14-7.62 6.0	Singlet Multiplet doublet	DMSO
12a	a.Vinylic protons (-C**H**=C)(SP2), b.Aromatic protons(Ar-**H**), c.**H**C=N	5.76,6.55 6.54-7.62 6.0	Singlet Multiplet doublet	DMSO

Table 5: Ferric Reducing Antioxidant Activity

Compound	IC_{50}	% Inhibition
1a	11 µg/ml	11.7
2a	14.9 µg/ml	38.5
3a	16.5 µg/ml	36.7
4a	14.6 µg/ml	15.14
5a	13.5 µg/ml	5.0
6a	11 µg/ml	4.55
7a	12.8 µg/ml	29
8a	14.5 µg/ml	45
9a	9.6 µg/ml	8.9
10a	22.6 µg/ml	27
11a	14.4 µg/ml	30
12a	14.3 µg/ml	33.5
Ascorbic acid	7 µg/ml	100

Table 6: Antibacterial activity

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Zone of inhibition (Diameter in Cm)																
Compound	**Bacillus subtilis**				**Staphylococcus aureus**				**Pseudomonas aeruginosa**				**Proteus vulgaris**			
	100 µg/ml	**150** µg/ml	**200** µg/ml	**250** µg/ml	**100** µg/ml	**150** µg/ml	**200** µg/ml	**250** µg/ml	**100** µg/ml	**150** µg/ml	**200** µg/ml	**250** µg/ml	**100** µg/ml	**150** µg/ml	**200** µg/ml	**250** µg/ml
1a	1.2	1.3	1.4	1.6	1.5	1.5	1.6	1.7	1.2	1.4	1.6	1.7	1.1	1.2	1.4	1.6
2a	1	1.1 l	1.2	1.3	1.1	1.2	1.2	1.4	1.1	1.3	1.4	1.6	1.2	1.2	1.3	1.5
3a	1.1	1.2	1.3	1.5	1.1	1.2	1.3	1.4	1.1	1.1	1.2	1.3	1.1	1.2	1.3	1.5
4a	1.1	1.2	1.2	1.5	1.1	1.2	1.3	1.4	1	1.1	1.2	1.3	1.1	1.1	1.2	1.3
5a	1.2	1.3	1.3	1.4	1.1	1.1	1.2	1.3	1.1	1.2	1.2	1.3	1.1	1.2	1.3	1.4
6a	1	1.1	1.2	1.3	1.1	1.2	1.3	1.4	1	1.1	1.2	1.4	1.2	1.3	1.4	1.5
7a	1.1	1.2	1.2	1.3	1.1	1.2	1.4	1.5	1.1	1.2	1.3	1.4	1.1	1.3	1.4	1.5
8a	1.1	1.1	1.2	1.4	1.2	1.3	1.4	1.5	1.2	1.2	1.3	1.4	1.2	1.3	1.4	1.5
9a	1.1	1.2	1.3	1.5	1.1	1.2	1.3	1.5	1.2	1.3	1.6	1.7	1.2	1.3	1.3	1.5
10a	1.1	1.3	1.4	1.6	1.6	1.6	1.7	2.0	1.3	1.5	1.8	2.0	1.1	1.2	1.4	1.6
11a	1.2	1.2	1.3	1.4	1.2	1.2	1.3	1.5	1.2	1.3	1.3	1.5	1.3	1.3	1.4	1.5
12a	1.3	1.4	1.6	1.7	1.3	1.4	1.6	1.8	1.2	1.4	1.6	1.7	1.3	1.4	1.5	1.6
Tetracycline	2	2	2.1	2.2	2.1	2.2	2.3	2.3	2	2.1	2.1	2.2	2	2.1	2.2	2.3
Chloramphenicol	2.1	2.2	2.3	2.4	2	2.1	2.2	2.3	2.1	2.2	2.3	2.4	2	2.1	2.2	2.3

Table 7: Antifungal Activity
Zone of inhibition (Diameter In Cm)

Compound	Aspergillums Niger				Pencillium chrysogenum				Pencillium notatum			
	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml	100 µ/ml	150 µ/ml	200 µ/ml	250 µ/ml
1a	0.9	0.9	1	1.1	1	1	1.1	1.1	1.1	1.1	1.2	1.3
2a	1.1	1.2	1.2	1.3	1.1	1.2	2	2.1	1.1	1.2	1.2	1.3
3a	1	1.1	1.2	1.3	1	1.1	1.2	1.2	1	1	1.1	1.2
4a	0.8	0.9	1	1.3	1	1.1	1.1	1.2	1	1	1.1	1.2
5a	0.9	1	1.1	1.1	0.9	1	1.1	1.1	1	1.1	1.2	1.2
6a	1.1	1.1	1.2	1.2	1	1.2	1.2	1.3	1	1.1	1.2	1.3
7a	0.9	1	1.1	1.1	1	1.1	1.1	1.2	1	1	1.1	1.1
8a	1.1	1.1	1.2	1.3	1.2	1.3	1.3	1.4	1.5	1.6	2.1	2.2
9a	0.8	0.9	1	1.2	0.9	1.1	1.2	1.3	1	1.2	1.3	1.4
10a	1.2	1.3	1.4	1.5	1.1	1.1	1.2	1.3	1.1	1.3	1.4	1.5
11a	1	1	1.1	1.2	1	1.1	1.2	1.3	1.1	1.1	1.2	1.2
12a	1.1	1.3	1.4	1.5	1.2	1.3	1.4	1.9	1.1	1.4	1.5	1.7
Fluconazole	1.7	1.8	1.9	2.0	1.6	1.8	1.9	2.0	1.8	1.9	2.0	2.1

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