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Synthesis and Antibacterial activity of substituted 2H-1,4-Pyridoxazin-3(4H)-one derivatives

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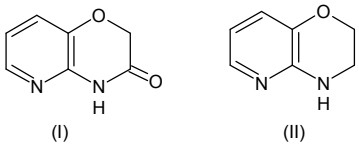
Abstract: 2*H*-1,4-pyridooxazin-3(4*H*)-one was synthesized by condensation of 2-amino-3-hydroxy-pyridine with chloroacetylchloride by using standard procedure . Esterification with ethylchloroacetate and further condensation with hydrazine hydrate produced hydrazide of 2*H*-1,4-pyridooxazin-3(4*H*)-one .Schiff base derivatives of 2*H*-1,4-pyridoxazin-3(4*H*)-one were synthesized by the acid catalyzed condensation 2-(3-oxo-2,3-dihydro-4*H*-1,4-pyridoxazin-4-yl)acetohydrazide with various benzaldehyde derivatives. Schiff base derivatives were characterized by FT-IR, ^{1}H-NMR. All the synthesized compounds were subjected to antimicrobial screening by cup plate method and by estimating the minimum inhibitory concentration by adopting the two-fold serial dilution technique.
Key words: Schiff base, Benzaldehyde derivatives, Antimicrobial activities, 2*H*-1,4-pyridoxazin-3(4*H*)-one.

INTRODUCTION:

2*H*-1,4-pyridooxazin-3(4*H*)-one(I)and 3,4-dihydro-2H-1,4 pyridoxazine (II) derivatives fused with other heterocyclic and aromatic ring system were reported to possess various activities such as anti-inflammatory, analgesic, antimicrobial analgesic, anticancer, etc.[1]

Figure:1



The isosteric replacement of other rings such as benzo, oxazolo has already been reported to possess antimicrobial activity. This study may help to achieve new chemical moieties that will help to minimize the drawbacks of conventional preparations such as penicillin, aminoglycosides and fluoroquinolones. Problems like super infection, narrow spectrum activity, toxic effect and bacterial resistance can be overcome by effective rational approach.[2]

Moreover, those skeletons can be utilized for the design of biologically active compounds, ranging from anti-inflammatory, analgesics, bactriostatic, fungistatic and MAO inhibitors etc.[3]

Benzoxazinoids analog of pyridoxazinone are natural pesticides that are abundant in sprouts of graminae including major agricultural crops such as wheat, maize and rye and also in few dicotyledonous. In plants they are stored as D-glycosides. Upon destruction of cellular structure the aglycones which are highly active are released by enzymatic degradation. Benzoxazinoids are part of the plant defense system against pests including insects, bacteria and fungi[4-6]

Virtanen et al[9-12] have isolated 2,4-dihydroxy-1,4-benzoxazine-3-one as a glycoside from rye plant. Since then a number of analogues of 1,4-benzoxazinone ring system have been studied for their chemotherapeutic activity. The most interesting

observation that ofloxacin molecule possesses the 1,4-benzoxazine ring like structure[13,14] prompted us to investigate this heterocyclic nuclei to ascertain if it would offer any advantage over the other known clinically used antimicrobial drugs.

In this study Schiff base of 2*H*-1,4-pyridoxazin-3(4*H*)-one derivatives were prepared and subjected to antimicrobial activity against different gram-positive and gram-negative bacteria.[15-18]

MATERIAL AND METHODS:

All the melting points reported were determined by open capillary tube method and are uncorrected. The synthesis and analytical studies of compounds were carried out using laboratory grade and analytical grade reagents as the case may be standard procedure or reported methods were followed with or without modification appropriately as and were required. The IR absorption spectra of the compounds were recorded on FTIR Bruker Tensor-27 model. The spectrometer values are expressed in cm^{-1} **.** The NMR spectra of the compounds were recorded in solvent – DMSO on an Bruker DRX-300 model using TMS as an internal reference. Chemical shift values are reported in δ (ppm).

EXPERIMENTAL SECTION:

Synthesis of 2*H*-1,4-pyridoxazin-3(4*H*)-one(1):

A mixture of 2-amino-3-hydroxy pyridine (mol. Wt. 109; 5g), dichloromethane (20ml) and triethylamine (4ml) is taken in round bottom flask, in ice cool condition, stir well using magnetic stirrer with simultaneous drop wise addition of ice cold chloroacetylchloride (3ml). The crude product is obtained by evaporating dichloromethane. Crude product was isolated and recrystallised with hot water and methanol in equal ratio. Yield 83%; M.P.206-208.Solubility – DMF, DMSO; Solvent system - chloroform: methanol (9.5:0.5); R_f value - 0.40; IR: 1700.85(C = O,str.), 3367(NH Str.), 1629(NH bend.), 744(Ar-H bend.), 3124.5C-H), 1195(C-O-C).NMR: NH (s,1H, 9.0), Ar –H(m, 3H, 8.176), CH_2(s, 2H, 4.74)

Synthesis of Ethyl (3-oxo-2,3-dihydro-4*H*-1,4-pyridoxazin-4-yl)acetate(2)[19]:

A mixture of 2*H*-1,4-pyridoxazin-3(4*H*)-one (mol. Wt. 149; 5g), sodium ethoxide (1.75gm) is taken in round bottom flask, stir well using magnetic stirrer, distill to remove ethanol, add N,N-dimethylformamide, then add ethyl chloroacetate (mol.wt. 122, 2.5ml) dropwise, reflux for at least 3 hours. The crude product is obtained by evaporating N,N-dimethylformamide by using rotary evaporator. Crude product was isolated and recrystallised with ethanol. The completion of reaction was monitored by running TLC. Yield: 57%; Melting point: 155-160 ^oC; Solubility: - methanol; Solvent system: - chloroform: methanol (9.5:0.5); R_f value: - 0.72; IR: 1724.89(C = O, ester), 1638(CONH , amide), 1406(C-N.), 744(Ar-H),2902, NMR: CH_3(t,3H,1.29), CH_2(q, 2H, 4.307), CH_2(s, 2H, 4.5), Ar-H(m,3H, 7-8.5)

Synthesis of 2-(3-oxo-2,3-dihydro-4*H*-1,4-pyridoxazin-4-yl)acetohydrazide(3)[20-21]:

A mixture of 2*H*-1,4-pyridoxazin-3(4*H*)-one ester (mol. Wt. 232; 12g) and hydrazine hydrate (1:1) is taken in round bottom flask, reflux for at least 6 hours. The crude product is obtained by evaporating excess hydrazine hydrate on water bath. The completion of reaction was monitored by running TLC. Yield: 60%; M.P: 140 ^oC; Solubility: chloroform; Solvent system: chloroform: methanol (9.5:0.5); R_f value: 0.96; IR: 1650, 1638.75(C = O), 3401.92(NH Str.),1361(C-N.), 3012.33(Ar-H str.), 744(Ar-H bend.), 2927(C-H), 1274(C-O-C), 1604(C=C Ar.).

NMR: CH_2(s, 2H, 4.5), NH_2(s, 2H, 10.5), Ar-H (m, 3H, 6.8-8.2)

General method for synthesis of 2-(3-oxo-2,3-dihydro-4*H*-1,4-pyridoxazin-4-yl)-*N*'-[1-(subtituted phenyl) methylidene] acetohydrazide(4a-4g)[22-24]:

2*H*-1,4-pyridoxazin-3(4*H*)-one acetohydrazide taken into a beaker and make soluble in chloroform using magnetic stirrer then add benzaldehyde derivatives in 1:1 ratio, add 1 or 2 drops of conc. HCl, stir on magnetic stirrer for about 2-4 hours, until solid separates, filter and allow to evaporate the remaining solvent. The completion of reaction was monitored by running TLC. Solubility: Dimethyl formamide; Solvent system: chloroform: methanol (9.5:0.5).

Schiff bases –

4a-.*N*'-[(4-hydroxyphenyl)methylidene]-2-(3-oxo-2,3-dihydro-4*H*-pyrido[3,2-*b*][1,4]oxazin-4-yl)aceto hydrazide

4b-*N*'-[(2-chlorophenyl)methylidene]-2-(3-oxo-2,3-dihydro-4*H*-pyrido[3,2-*b*][1,4]oxazin-4-yl)aceto hydrazide

4c-*N*'-[(2-hydroxyphenyl)methylidene]-2-(3-oxo-2,3-dihydro-4*H*-pyrido[3,2-*b*][1,4]oxazin-4-yl)aceto hydrazide

4d-*N*'-{[4-(dimethylamino)phenyl]methylidene}-2-(3-oxo-2,3-dihydro-4*H*-pyrido[3,2-*b*][1,4]oxazin-4-yl)acetohydrazide
4e-.*N*'-(furan-3-ylmethylidene)-2-(3-oxo-2,3-dihydro-4*H*-pyrido[3,2-*b*][1,4]oxazin-4-yl)acetohydrazide
4f-*N*'-[(4-chlorophenyl)methylidene]-2-(3-oxo-2,3-dihydro-4*H*-pyrido[3,2-*b*][1,4]oxazin-4-yl)aceto hydrazide
4g-2-(3-oxo-2,3-dihydro-4*H*-pyrido[3,2-*b*][1,4]oxazin-4-yl)-*N*'-(phenylmethylidene)acetohydrazide.

Figure:2 Scheme of Synthesis

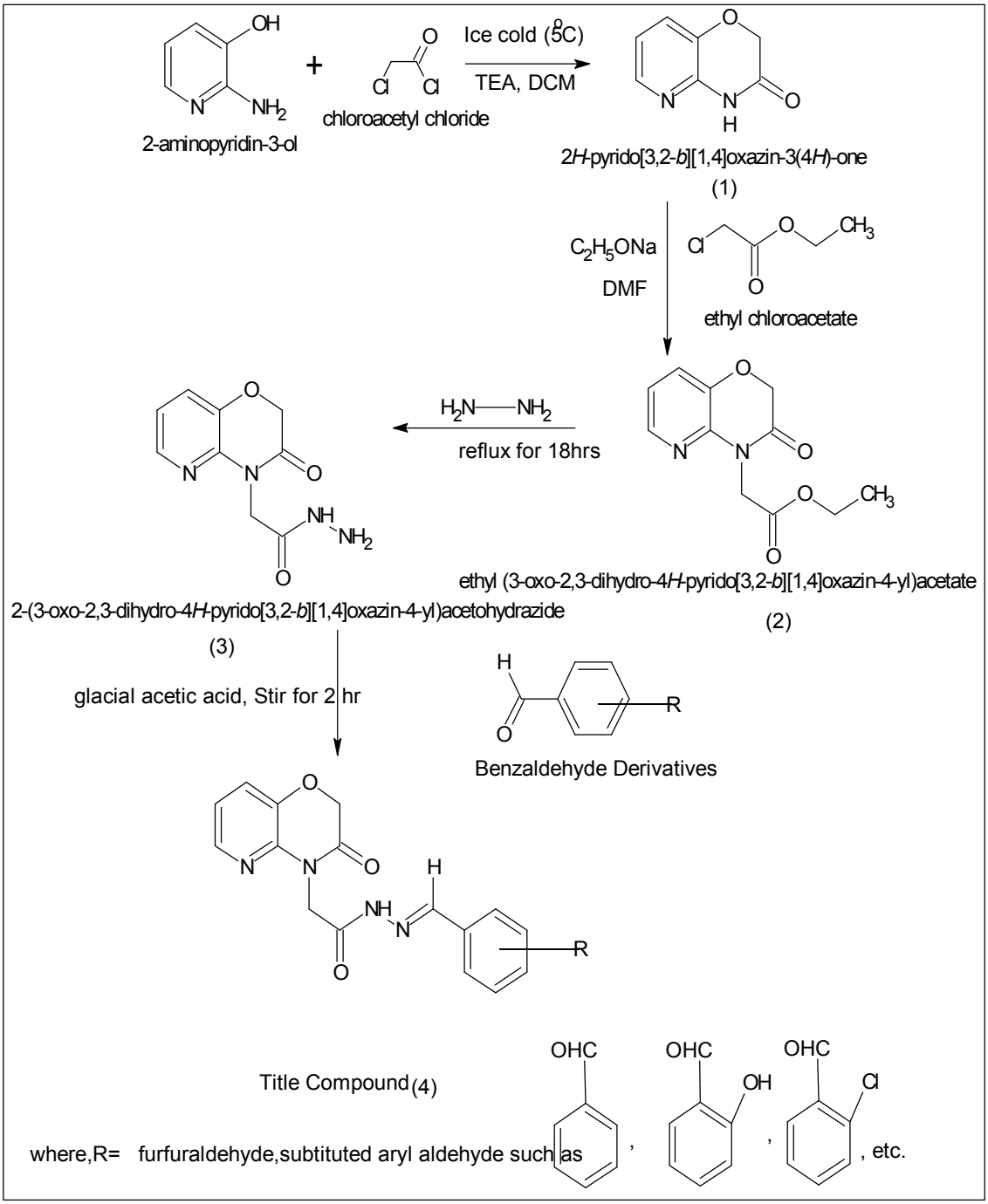


Table 1.- IR and 1HNMR spectral data of synthesized title compounds

Comp No.	Wave Number{cm^{-1}}	Chemical Shift (δ value)
4a.	3208.27(OH str.), 2921.167(CH_2 str.) , 1699.412(C=O str), 1595.56(C=N), 758.66(CH bend.)	9.925(s,1H, OH),4.41(s, 2H, CH_2), 4.93(s, 2H, CH_2), 6.8-8.553(m, 7H, Ar-CH), 10.56(s, 1H, NH), 8.946(s, 1H, CH).
4b.	1642(C = O), 3124(NH Str.), 1504(NH bend.),1600 (C=N.), 3054.61(Ar-H),1403(C-H Bend), 7412.67(C-Cl), 1060(C-O-C)	4.97(s, 2H, CH_2), 6.791-8.654(m, 7H, Ar-CH), 10.569(s, 1H, NH), 9.435(s, 1H, CH), 4.46(s, 2H, CH_2),
4c.	3334.23(OH str.)1700.79(C = O), 3128(NH Str.), 1636.79(NH bend.), 1196 (C-N.), 3128,873(Ar-H), 1640 (C= CAr) 2909.46(CH str.),1401(C-H), 1049(C-O-C)	9.71(s,1H,OH),4.45(s, 2H, CH_2), 6.87-8.15(m, 7H, Ar-CH), 10.5 (s, 1H, NH), 8.562(s, 1H, CH),4.95(s, 2H, CH_2),
4d.	1721(C = O), 3350(NH Str.), 1555(NH bend.), 1382(C-N.), 3059,742(Ar-H), 2902,1492(C-H), 1195(C-O-C),1039(C-Cl)	4.431(s, 2H, CH_2), 6 -8.431(m, 7H, Ar-CH), 10.020(s, 1H, NH), 8.601(s, 1H, CH), 2.49(s, 6H, CH_3)
4e.	1679(C = O), 1082(C-N.), 3051, 744(Ar-H), 1620(C= C Ar), 3051(C-H Str.), 1477(C-H bend.), 1214(C-O-C), 1611(C=C Ar.).	4.947(s, 2H, CH_2), 6.85-8.01(m, 8H, Ar-CH), 9.064(s, 1H, NH)
4f.	1700.47(C = O), 3391(NH Str.), 1633.36(NH bend.), 1264(C-N.), 3033,803(Ar-H), 2942(C-H Str.), 1433(C-H Bend), 1063(C-O-C).	4.44(s, 2H, CH_2), 6.86-8.11(m, 8H, Ar-CH), 10.8(s, 1H, NH),
4g.	1655(C = O), 3324(NH Str.), 1591(NH bend.), 1190(C-N.), 3050,765(Ar-H), 2885(C-H Str.), 1400(C-H Bend), 3440(O-H), 1100(C-O-C).	4.534(s, 2H, CH_2), 6.854-8.540(m, 8H, Ar-CH), 10.442(s, 1H, NH), 4.634(s, 2H, CH_2)

Table 2.- The physico-chemical data of synthesized title compounds:

Compd.	Mol. formula	Mol. Wt.	R_f	Color (Appearance)	M.P. (^oC)
4a.	$C_{16}H_{14}N_40_4$	326.31	0.91	Light brown (crystalline)	190
4b.	$C_{16}H_{13}ClN_40_3$	344.75	0.89	Pale yellow (crystalline)	201
4c.	$C_{16}H_{14}N_4O_4$	326.31	0.92	Pale orange (crystalline)	215
4d.	$C_{18}H_{19}N_5O_3$	353.37	0.94	Light brown (crystalline)	174
4e.	$C_{14}H_{12}N_4O_4$	300.27	0.88	Pale brown (crystalline)	143
4f.	$C_{16}H_{13}ClN_4O_3$	344.74	0.91	Black (crystalline)	160
4g.	$C_{16}H_{14}N_4O_3$	310.30	0.78	Off white (crystalline)	158

ANTI BACTERIAL ACTIVITY[25-26]:

For determining antibacterial activity, compounds were dissolved in DMF. Further dilutions of the compounds and standard drug (ciprofloxacin) in the test medium were furnished at the required quantity of 500ppm which is minimum inhibitory concentration were determine using the method two-fold serial dilution technique. In order to ensure that the solvent had no effect on bacterial growth, a control test also performed containing inoculated broth supplemented with only DMF at the same dilution used in our experiments and found inactive in culture media.

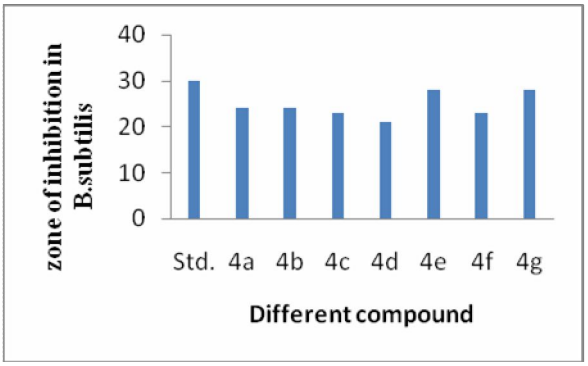
Antibacterial assay

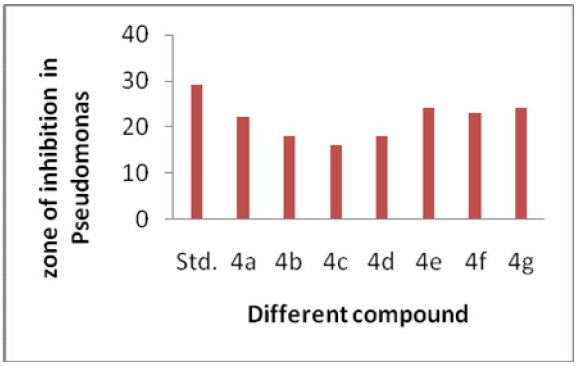
Sterile nutrient broth plates were prepared by pouring the sterile agar into petri dishes in aseptic condition. 0.1ml of each standardized test organism spread into agar plates. Holes were prepared by using a sterile box of diameter 6mm.The test drugs as well as the standard drug (500ppm) and the solvent control were placed in each hole separately. Then the plates were maintained at 4^0C for 1hr to allow the diffusion of solution into medium. All bacterial plates were incubated at 37^0C for 24 hr. The zones of inhibition were measured in mm.

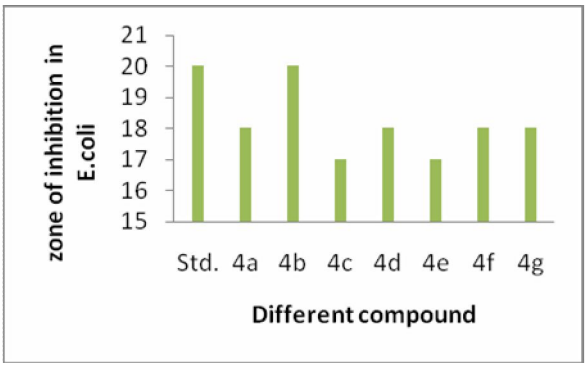
Table 3 - Observation table for microorganism zone of inhibition:

Compounds	Microrganisms			
	Gram-positive	Gram-negative		
	B.subtilis	E.coli	Pseudomonas	K.pneumoniae
Ciprofloxacin	30	20	29	25
4a	24	18	22	18
4b	24	20	18	18
4c	23	17	16	17
4d	21	18	18	17
4e	28	17	24	18
4f	23	18	23	17
4g	28	18	24	19

Figure:3, Histogram showing zone of inhibition of standard and synthesized derivatives







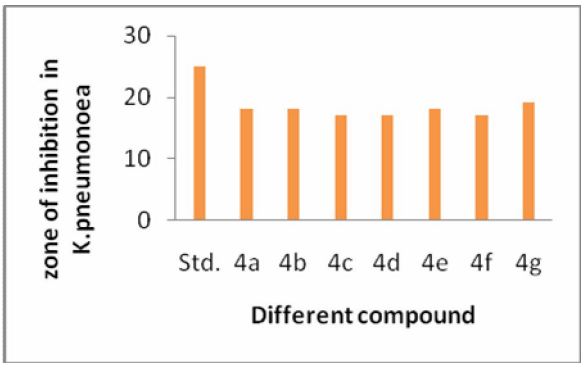
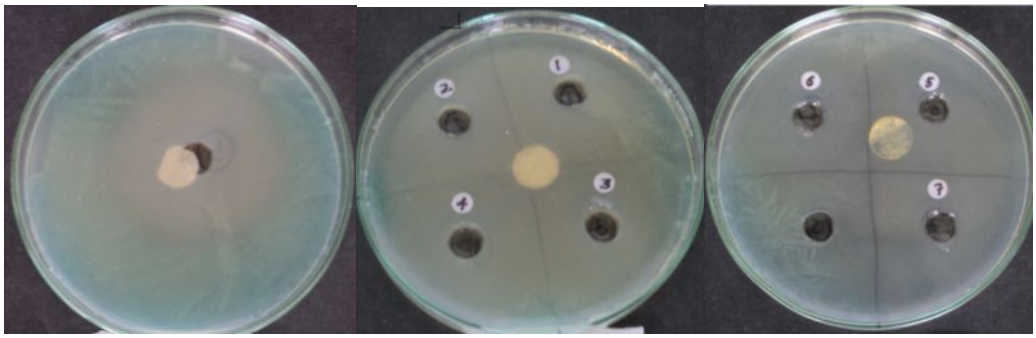
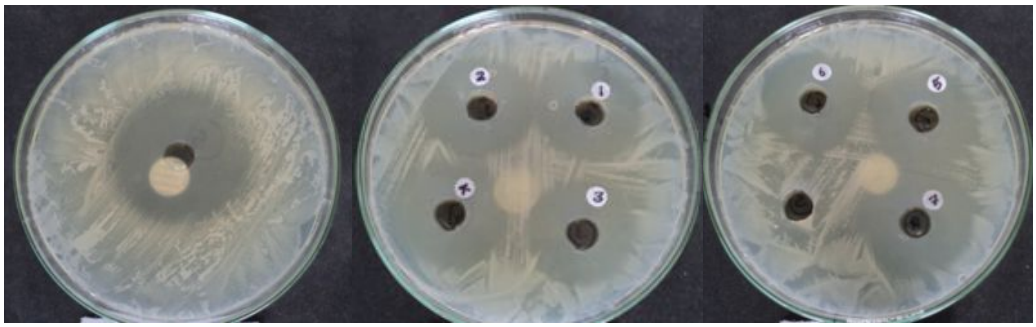


Figure : 4, Zone of inhibition of standard(ciprofloxacin) and test compounds:



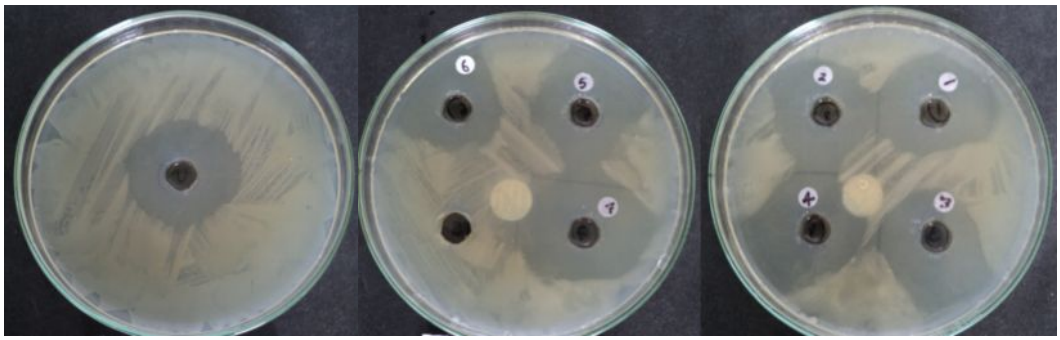
Standard **Test compds. (4a-4d)** **Test compds. (4e-4g)**

Pseudomonas



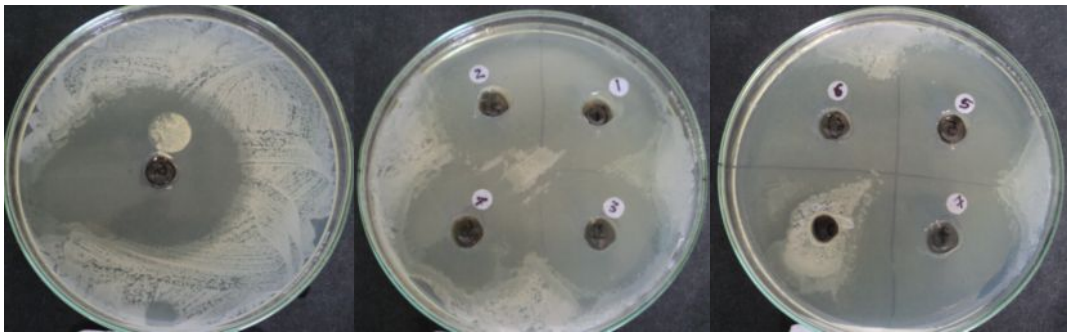
Standard **Test compds. (4a-4d)** **Test compds. (4e-4g)**

K.pneumoniae



Standard **Test compds. (4e-4g)** **Test compds. (4a-4d)**

E-coli



Standard **Test compds. (4a-4d)** **Test compds. (4e-4g)**

Bascillus

Table 4- Observation table of percentage zone of inhibition for Gm+ve and Gm-ve microbial strains:

Compounds	Microrganisms			
	Gram-positive	Gram-negative		
	B.subtilis	E.coli	Pseudomonas	K.pneumoniae
Ciprofloxacin	100	100	100	100
4a	80	90	75.8	72
4b	80	100	62	72
4c	76.6	85	55.1	68
4d	70	90	62	68
4e	93.3	85	82.7	72
4f	76.6	90	79.3	68
4g	93.3	90	82.7	76

RESULTS & DISCUSSION:

The attempt to synthesize Schiff bases of 2*H*-1,4-pyridoxazin-3(4*H*)-one were successfully carried out as per the scheme mentioned. The entire synthesized compounds are primarily characterized by running T.L.C. and melting point analysis.

The structures of the synthesized compounds are confirmed by I.R.and NMR spectral data.

All the compounds shows good activity against E. Coli, B. subtilis, P. aeruginosa, K. pneumonia. Compound 4b shows excellent activity against E. Coli, 4e and 4g shows excellent activity against B. subtilis and P. aeruginosa, 4a, 4b, 4e, 4g shows excellent activity against K. pneumonia in comparison to standard drug ciprofloxacin.

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