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Novel Hydrazone derivates as the potent Mosquito larvicidal and anti-bacterial agents and its synthesis

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ABSTRACT

The present deals with the study of series of novel 1-(c-phenyl) ethanone /c-benzaldehyde (5a-j) obtained by refluxing pyridine-4-carbohydrazide (3) with various substituted aldehydes and ketones (4).the structures of newly synthesized compounds were confirmed by using spectroscopic analysis. The entire synthesized compound was screened for Mosquito larvicidal and anti-bacterial activity studies which showed good to moderate activity**.**

Keywords: Hydrazones; Mosquito larvicidal; Anti-bacterial.

1. INTRODUCTION

Mosquitoes are the well known vectors which are able to carry many disease caused by bacteria, viruses or parasite. It transmits the disease by injecting the saliva and anti coagulants through its bite. It becomes cumbersome to control the adult mosquitoes but they can be easily monitored and controlled during the larval stage.

Hydrazones are important class of pharmacologically active heterocyclic compounds with a wide range of biological activity. They are essential building blocks for various organic synthesis, with the general formula R^1R^2C-N–NHR^3(R = alkyl or aryl groups).In particular, these organic molecules have been received considerable attention as Anti-bacterial [1-4], Anti-cancer [5], Antitubercular [6], Antifungal [7],Antiangiogenic [8], Antimicrobial [9], Antidepressant [10], Antioxidant [11], Analgesic [12], Trypanocidal [13], antibiotic activities [14], anti-metastatic potential [15], Anti-parasite [16] and DNA interaction , Cytotoxic, and radical scavenging [17].

Enthusiased by the broad biological activities of the hydrazone derivatives, in this work we now report the successful synthesis of a series of 1-(c-phenyl) ethanone /c-benzaldehyde (5a-j).

2. EXPERIMENTAL SECTION

Melting points of the compounds (5a-j) were determined. The purity of novel compounds was confirmed by observing single spot on TLC plate, Merck silica gel 60 F_{254} coated alumina plates. The structures of these novel compounds (5a-j) were confirmed through spectral studies. The ^{1}H-NMRand ^{13}C-NMR spectra was recorded on Brucker AMX-400(400MHz) spectrometer using $CdCl_3$-d as solvent and TMS as the internal standard. The IR spectra (cm^{-1}) were recorded on a Shimadzu-FTIR 577 infrared spectrometer in KBr pellets. The mass spectra were recorded on Perkin –Elmer 018444Y, triple quadrapole LC/MS spectrometer. The synthesized novel compounds showed the molecular ion peak (m/z) equivalent to their molecular weight.

2.1. 1-(c-phenyl) ethanone /c-benzaldehyde (5a-j)

The novel series of 1-(c-phenyl) ethanone /c-benzaldehyde (44a-j) were synthesized according to the procedure reported in the literature [18-20]. The equimolar ratio of pyridine-4-carbohydrazide (0.001mol) (3) was treated with various substituted aldehydes and ketones (4) (0.001mol) were stirred in for 8hrs at room temperature in DMF .the reaction mixtures were poured into crushed ice .the solid mass separated were filtered and recrystallized from ethanol to afford novel analytical samples (5a-j) (Scheme 1).

2.2. *N*'-[(1*Z*)-1-(anthracen-9-yl) ethylidene] pyridine-4-carbohydrazide (5a)

IR (KBr, Cm^{-1}): 3349.2(>N-H stretch), 3027.5(>C-H stretch, of CH_3), 1662.9 (>C=0) and 1539.3, (>C=N).^{1}H-NMR: 1. 2.369((3H, s, methyl

group attached to C=N), 8.774 (2H, d, J=8.8 pyridine moiety), 11.130 (1H, s, NH adjacent to carbonyl group), 7.799(2H, d, J=8.4 pyridine moiety), 7.224-8.021(9H, m, anthracene moiety).^{13}C-NMR: 19.001(1C atom of methyl group) ,156.170, 153.903, 141.296, 130.247, 127.076 (5C atoms pyridine moiety), 127.337, 127.276, 127.044, 126.883, 125.976, 124.567, 122.145, 121.943, 121.456, 120.944, 120.758, 119.672, 119.786, 118.156, 117.923 (15C atoms of anthracene moiety), 162.915 (>C=0 adjacent to pyridine moiety), 150.083 (1C atom of C=N next to carbonyl group). LC-mass, [M$^+$+1], (m/Z): 339.07.

2.3. *N*'-[(1*Z*)-1-(2-fluoro-4-methoxyphenyl) ethylidene] pyridine-4-carbohydrazide (5b)

IR (KBr, Cm^{-1}): 3235.6(>N-H stretch), 3024(>C-H stretch, of CH_3), 1653.3 (>C=0), 1538.2, (>C=N), and 1006.7(-C-F stretch). ^{1}H-NMR: 2.785(3H, s, methyl group attached to C=N), 3.768(3H, s, methoxy attached to 2-fluoro benzene) ,8.774(2H, d, J=8.8 pyridine moiety), 11.121 (1H, s, NH adjacent to carbonyl group), 7.885 (2H,d,j=8.8-2-fluoro,4-methoxy benzene),7.765 (2H, d, J=8.4 pyridine moiety), 8.004 (1H,s,2-fluoro,4-methoxybenzene).^{13}C-NMR: 55.109, 18.654(2C atoms of methoxy and methyl group) ,156.970, 154.703, 140.796, 131.237, 127.076 (5C atoms pyridine moiety) ,127.044, 121.943, 120.944, 120.758, 118.156, 117.923 (6C atoms of 2-fluoro, 4-methoxy phenyl moiety), 163.005(>C=0 adjacent to pyridine moiety), 150.083 (1C atom of C=N next to carbonyl group moiety). LC-mass: [M$^+$+1], (m/Z): 287.1/ 288.1.

2.4. *N*'-{(*Z*)-[4-chloro-3-(trifluoromethyl) phenyl] methylidene} pyridine-4-carbohydrazide (5c)

IR (KBr, Cm^{-1}): 3335.6(>N-H stretch), 1975.1(>C-H stretch,), 1643.3 (>C=0), 1528.2, (>C=N), 752.32 (>C-Cl stretch) and 1016.7(>C-F stretch).^{1}H-NMR: 8.123(1H,s,methyl group attached to C=N),7.456,7.487 ,(2H ,d, J=8.8 -3-fluryl ,4-chloro benzene moiety),7.806(2H,d,J=8.4 pyridine moiety) ,11.540 (1H,s,NH adjacent to carbonyl group),8.774(2H,d,J=8.8 pyridine moiety),8.654(1H,s,3-fluryl ,4-chloro benzene moiety).^{13}C-NMR: 60.913(1C atom of fluryl group) ,158.170, 155.703, 141.796, 137.237, 126.076(5C atoms pyridine moiety) ,127.044, 121.943, 120.944, 120.758, 118.156, 117.923 (6C atoms of 4-chloro,3-fluryl phenyl moiety), 162.615 (>C=0 adjacent to pyridine moiety), 150.093(1C atom of C=N next to carbonyl group moiety). LC-mass: [M$^+$+1], (m/Z):328.12/325.43.

2.5. *N*'-[(*Z*)-(3-hydroxy-4-methylphenyl) methylidene] pyridine-4-carbohydrazide (5d)

IR (KBr, Cm^{-1}): 3348(>N-H stretch), 3024(>C-H stretch, of CH_3), 1668 (>C=0), 1533, (>C=N), and (OH stretch) ^{1}H-NMR: 2.439(3H,s,methyl group attached to 3-hydroxy benzene), 8.123(1H,s,methyl group attached to C=N) ,7.446,7.434 ,(2H ,d, J=8.8 -3-hydroxy,4-methyl benzene moiety),7.799(2H,d,J=8.4 pyridine moiety) ,11.230 (1H,s,NH adjacent to carbonyl group),8.774(2H,d,J=8.8 pyridine moiety), 6.788(1H,s,hydroxyl group attached to 4-methyl benzene moiety),8.004 (1H,s, 3-hydroxy,4-methyl benzene moiety). ^{13}C-NMR: 18.684 (1C atom of methyl group), 156.970, 154.703, 140.796, 131.237, 127.076 (5C atoms pyridine moiety) ,127.044, 122.943, 120.844, 120.658, 119.156, 117.823 (6C atoms of 3-hydroxy, 4-methyl phenyl moiety), 163.073(>C=0 adjacent to pyridine moiety), 150.084 (1C atom of C=N next to carbonyl group moiety). LC-mass:[M$^+$+1],(m/Z): 255.2.

2.5. *N*'-[(1*Z*)-1-(3-bromo-4-fluorophenyl) ethylidene]pyridine-4-carbohydrazide (5e).

IR (KBr, Cm^{-1}): 3339.2 (>N-H stretch), 3029.5 (>C-H stretch, of CH_3), 1652.9 (>C=0), 1539.3, (>C=N), 576.8 (>C-Br) and1017.5 (>C-F). ^{1}H-NMR:2.439 (3H,s,methyl group attached to C=N),7.566(2H ,d, J=8.8 pyridine moiety),7.804(3H,m,3-Br,4-F-benzene moiety),11.127(1H,s,NH adjacent to carbonyl group) ,8.778(2H,d,J=8.8 pyridine moiety). ^{13}C-NMR: 18.385 (1C atom of methyl group), 157.170, 154.703, 140.796, 131.237, 127.076(5C atoms pyridine moiety), 127.044, 121.943, 120.944, 120.758, 118.156, 117.923 (6C atoms of 3-bromo, 4-fluoro phenyl moiety), 162.715 (>C=0 adjacent to pyridine moiety), 150.083(1C atom of C=N next to carbonyl group moiety). LC-mass: [M$^+$+1], (m/Z):336.01/333.98.

2.6. *N*'-[(1*Z*)-1-(2, 4-dichloro-5-fluorophenyl) ethylidene]pyridine -4-carbohydrazide (5f).

IR (KBr, Cm^{-1}): 3348 (>N-H stretch), 1668 (>C=0), 1533 (>C=N), 721,748 (>C-Cl stretch) and 1017.5 (>C-F stretch). ^{1}H-NMR:2.369((3H,s,methyl group attached to C=N), 7.566(2H ,d, J=8.8 pyridine moiety),7.904,7.804(2H,s,2,4-dichloroBr,5-F-benzene moiety), 7.990(2H,d,J=8.8 pyridine moiety),11.230(1H,s,NH adjacent to carbonyl group),8.778(2H,d,J=8.8 pyridine moiety).^{13}C-NMR: 18.654(1C atom of methoxy group), 156.970, 154.703, 140.796, 131.237, 127.076 (5C atoms pyridine moiety) 127.044, 121.943, 120.944, 120.758, 118.156, 117.923 (6C atoms of 2, 4-dichloro, 5-fluoro phenyl moiety), 163.005(>C=0 adjacent to pyridine moiety) ,150.083 (1C atom of C=N next to carbonyl group moiety). LC-mass: [M$^+$+1], (m/Z): 326.5/324.2.

2.7. *N*'-[(*Z*)-(3,4-dichloro-2-fluorophenyl) methylidene]pyridine-4-carbohydrazide (5g).

IR (KBr, Cm^{-1}): 3335.6 (>N-H stretch), 3045 and 1975.1cm^{-1} (>C-H stretch,), 1643.3 (>C=0), 1528.2 (>C=N), 748.38, 752.32 (>C-Cl stretch) and 1016.7 (>C-F stretch).

^{1}H-NMR: 8.219(1H,s,methyl group attached to C=N),7.446,7.568 ,(2H ,d, J=8.8 -3,4 dicloro,2-fluoro benzene moiety),7.990(2H,d,J=8 pyridine moiety) ,11.230 (1H,s,NH adjacent to carbonyl group),8.778(2H,d,J=8.8 pyridine moiety). ^{13}C-NMR:157.176, 154.709 ,139.840 ,133.088 ,131.210 (5C atoms pyridine moiety) ,130.674 ,129.059 ,128.985 ,121.949 ,120.156 ,118.156 (6C atoms of 3, 4-dichloro, 2-fluoro phenyl moiety), 162.896(>C=0 adjacent to pyridine moiety) 150.098(1C atom of C=N next to carbonyl group moiety). LC-mass: [M^{+}+1], (m/Z):311.9/310.0.

2.8. *N*'-[(*Z*)-(2,3,5-trichlorophenyl) methylidene]pyridine-4-carbohydrazide (5h)

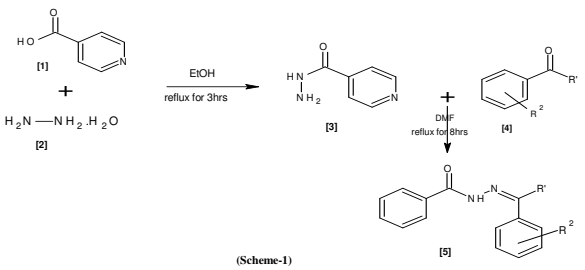
IR (KBr, Cm^{-1}): 3342.3 (>N-H stretch), 3073.47, 2960.44, 2021.1 (>C-H stretch,), 1658.5 (>C=0), 1528.2 (>C=N) and 721, 747.38 ,751.6 (>C-Cl stretch) ^{1}H-NMR: 8.109 (1H,s,methyl group attached to C=N),7.446,7.434 ,(2H ,d, J=8.8 -3,4 dicloro,2-fluoro benzene moiety),7.799(2H,d,J=8.4 pyridine moiety) ,11.230 (1H,s,NH adjacent to carbonyl group),8.774(2H,d,J=8.8 pyridine moiety). ^{13}C-NMR: 157.176, 154.709, 140.409, 133.088, 131.210 (5C atoms pyridine moiety), 130.674, 128.059, 127.985, 121.949, 120.156, 116.156 (6C atoms of 2, 3, 5-trichloro phenyl moiety), 162.896 (>C=0 adjacent to pyridine moiety) 150.098 (1C atom of C=N next to carbonyl group moiety).LC-mass: [M^{+}+1], (m/Z):328.0/324.1.

2.9. *N*'-[(1*Z*)-6-methoxy-1*H*-inden-1-ylidene] pyridine-4-carbohydrazide(5i)

IR (KBr, Cm^{-1}): 3348(>N-H stretch), 3024(>C-H stretch, of CH_3), 1668 (>C=0), 1533, and (>C=N) ^{1}H-NMR: 3.768(3H, s, methoxy attached to 1-indene moiety), 6.998-7.342(5H, m, 4-methoxy indene), 8.774(2H, d, J=8.8 pyridine moiety), 11.230 (1H, s, NH adjacent to carbonyl group), 7.799(2H, d, J=8.4 pyridine moiety).^{13}C-NMR: 55.109 (1C atom of methoxy group), 156.970, 154.703, 140.796, 131.237, 127.076 (5C atoms pyridine moiety), 127.337, 126.883, 125.976, 124.567, 121.943, 120.944, 119.786, 118.156, 117.923 (9C atoms of 1*H*-inden-5-yl methyl ether moiety), 163.005 (>C=0 adjacent to pyridine moiety), 150.065 (1C atom of C=N next to carbonyl group moiety). LC-mass: [M^{+}+1], (m/Z):381.5.

2.10. *N*'-[(*Z*)-(2-bromo-4,5-dimethoxyphenyl) methylidene]pyridine-4-carbohydrazide (5j).

IR (KBr, Cm^{-1}): 3339.2(>N-H stretch), 3029.5(>C-H stretch, of CH_3), 1652.9 (>C=0),1539.3, (>C=N), and 576.8 (>C-Br stretch) .^{1}H-NMR: 3.768,3,887(6H, s, methoxy attached to 1-indene moiety),8.123(1H,s,methyl group attached to C=N),7.456,7.487 (2H ,s, 2-bromo ,4,5-dimethoxy benzene moiety),7.806(2H,d,J=8.4 pyridine moiety) ,11.540 (1H,s,NH adjacent to carbonyl group),8.774(2H,d,J=8.8 pyridine moiety). ^{13}C-NMR: 55.109, 55.186 (2C atoms of 2methoxy groups), 155.970, 153.703, 141.796, 131.237, 127.076 (5C atoms pyridine moiety), 127.337, 126.883, 125.976, 124.567, 121.943, 120.944, 119.786, 118.156, 117.923 (9C atoms of 1*H*-inden-5-yl methyl ether moiety), 163.026 (>C=0 adjacent to pyridine moiety), 150.063 (1C atom of C=N next to carbonyl group moiety). LC-mass: [M^{+}+1], (m/Z):364.01.



Scheme - 1: The synthesis of pyridine-4-carbohydrazide (3).

Table I- Characterization data 1-(c-phenyl) ethanone /c-benzaldehyde (5a-j)

Sample	R 1	R 2	Mol. Formula	M. W	M. P ^{0}C
5a	CH_3	$C_{14}H_{10}$	$C_{22}H_{17}N_3O$	190-193	339.38
5b	CH_3	2-F,4-OCH_3 -(C_6H_5)	$C_{15}H_{14}FN_3O_2$	137-144	287.288
5c	H	3-CF_3,4-Cl (C_6H_5)	$C_{14}H_9ClF_3N_3O$	154-159	327.688
5d	H	3-OH,4- CH_3 -(C_6H_5)	$C_{14}H_{13}N_3O_2$	119-121	255.27
5e	CH_3	3-Br,4- F-(C_6H_5)	$C_{14}H_{11}BrFN_3O$	113-116	336.150
5f	CH_3	2,4-$(Cl)_2$,5-F -(C_6H_5)	$C_{14}H_{10}Cl_2FN_3O$	168-172	326.153
5g	H	3,4-$(Cl)_2$,2-F -(C_6H_5)	$C_{13}H_8Cl_2FN_3O$	186-190	312.126
5h	H	2,3,5-$(Cl)_3$- (C_6H_5)	$C_{13}H_8Cl_3N_3O$	139-141	328.58
5i		$C_{10}H_{10}O$	$C_{16}H_{13}N_3O_2$	156-162	279.29
5j	H	2-Br-4,5 $(OCH_3)_2$ -(C_6H_5)	$C_{15}H_{14}BrN_3O_3$	163-169	364.193

2.11. CHEMISTRY

The synthesis of the target compounds is depicted in the (scheme-1) and synthesized according to the procedure reported in the literature[44-49] .The series of novel compounds was prepared in an excellent yield by the reaction of equimolar mixture of pyridine-4-carboxylic acid (1) and hydrazine hydrate (2) which is refluxed for 3hours in ethanol medium. The resultant pyridine-4-carbohydrazide (3) was treated with equimolar ratio of various substituted aldehydes and ketones (4) to yield a series of (5a-j) in DMF. The Characterization data is given in table 1.

2.12. Biological assay

Anti-bacterial activity

The antibacterial activity of novel compounds *N*'-[(*Z*)-(2,3,5-trichlorophenyl)methylidene] pyridine-4-carbohydrazide (45a-j) was carried out *in vitro* by Disc Diffusion Method (Zone inhibition test) using the two gram positive bacteria, *Staphylococcus aureus(MTCC-7443)* and *Bacillus subtilius(MTCC-441)* viz., and two gram negative bacteria ; viz., *Escherichia coli(MTCC-725) and Klebsiella pneumonia(MTCC-1739)*viz.,. The reference standard used for screening is Ciprofloxacin, an antibiotic drug. The microorganisms for the screening were collected from Institute of Microbial Technology (IMTECH), Chandigarh, India.

The colonies of the microbial strains were inoculated on nutrient agar plates with the help of sterile loop and visually adjusted the turbidity with broth to broth to match that of 0.5 McFarland standards. The excess of the inoculums was removed by rotating the sterile swab dipped in to the inoculum against the wall of the tube against it approximately 60^{o}C between streaking, the procedure is repeated three times to ensure even distribution. After 3 mins sterile discs of the size 6mm diameter were aseptically impregnated with the test compounds at a concentration 50µg/ml.The plates were incubated at 37^{0}C for 24h.The compounds that produce distinct circular zones of inhibition around the discs .the diameter of clear zone indicate the anti-bacterial activity.

The antibacterial activity of the synthesized target compounds were revealed that these compounds showed to moderate to good activity .out of which the compounds 5e , 5g and 5h showed sensitivity towards both the strains of bacteria used, where as 5c and 5j were found to show pronounced activity towards gram negative and gram positive bacterial strains respectively. The results are tabulate in table 2.

Larvicidal activity in Mosquito

Larvicidal bioassay for the target compound was carried out according to the standard protocol conducted in accordance with the WHO (World Health Organization, 1981). The analysis was made using late third or early fourth instar larvae. The experiment was performed by using 250-mL glass beaker, containing 1 mL of each of the test chemicals and 99 mL distilled water along with negative control containing 1 ml acetone with 0.001% Tween -80 , kept with each set of the experiment. Concentration ranging from 50 ppm to 800 ppm were prepared from the 1% stock solution (1% acetone + 0.001% Tween s of the 80).After 24 hours of incubation at room temperature the mortality of the larvae used for the experiment was determined. The experiment was carried out in triplets for each of the target compound and their mean (±SD) values were taken. The Median lethal concentration (LC_{50})

Table - 2: Anti-bacterial activity of 1-(c-phenyl) ethanone /c-benzaldehyde (45a-j) against Gram positive and Gram negative Bacteria by disc diffusion method (ZOI test)

Control/Sample	Zone of inhibition (mm)			
	S. aureus	*B.subtilis*	*E. coli*	*K.pneumonia*
5a	19	12	18	14
5b	18	22	18	19
5c	9	05	30	24
5d	10	16	10	12
5e	23	21	31	22
5f	24	29	31	09
5g	22	28	30	21
5h	24	29	31	22
5i	10	15	12	13
5j	12	16	19	17
Ciprofloxacin	26	30	32	28m

[A]Mean values of 3 trails, '0' indicates no sensitivity (zone of inhibition <7mm), Ref.Std: Ciprofloxacin **(10 µg/disc)**

Table - 3: Larvicidal activity of 1-(c-phenyl) ethanone /c-benzaldehyde (45a-j) against three mosquito vectors *A. aegypti, C. quinquefasciatus, A. stephensi*

Test compounds	*A. aegypti*		*C. quinquefasciatus*		*A. stephensi*		Average LC-50
	LC-50	LC-90	LC-50	LC-90	LC-50	LC-90	
Control	-		-	-	-	-	-
5a	3.3	4.9	2.9	4.2	3.4	5.1	3.2
5b	2.9	4.3	2.2	3.4	3.2	4.7	2.76
5c	3.4	5.1	3.6	5.3	3.5	5.1	3.5
5d	2.8	4.3	3.2	4.8	2.6	4.0	2.86
5e	2.7	4.1	3.1	4.7	2.9	4.4	2.9
5f	2.9	4.4	2.5	3.8	3.1	4.8	2.83
5g	3.2	4.7	2.9	4.3	2.6	4.0	2.9
5h	2.6	3.9	2.4	3.7	2.7	4.2	2.56
5i	3.1	4.8	3.5	5.2	3.2	4.6	3.26
5j	3.2	4.9	2.9	4.5	3.5	5.2	3.2
Ref.std. (Temephos)	0.019	0.0612	0.016	0.049	0.017	0.078	-

with 95% confidence limit was calculated using Abbott's formula (1925) and Log probit analysis, and results are expressed as ppm. The results obtained are tabulated in table 3 and figure 1.

The synthesized novel compounds were tested against the three species of mosquito, among which most of the compounds were sensitive towards the larvae of *C. quinquefasciatus and* indicated by less LC-50 values compared to those of the other two species. Taken into account of LC-50 values <2.2mg/ml as the substantial activity, among the test compounds, the compound 5h is found to be more effective against all the three bacterial strains *A. Aegypti, C. quinquefasciatus and A. stephensi.*5d and 5e are effective against *A. Aegypti and A. stephensi.where as the target compound 5b is more effective against C. quinquefasciatus.* The average LC-50 values indicate that 5b is more effective followed by 5h, 5d and 5e. Thus, among the test compounds, 5b can be considered as a potential broad spectrum larvicidal agent. If this compound is found to be less toxic to non-target organisms upon other toxicity studies, it may be recommended as a larvicidal agent. Reference standard temephos exhibited very high larvicidal activity. However, it has greater limitations in terms of its toxic effects on non-target organisms. Therefore, efficacy of the test compounds cannot directly be compared (hence, in the bar diagram, it is not shown).

3. RESULTS AND DISCUSSION

All the reported compounds in the present study were characterized by using the spectroscopic analysis.

IR spectrum of compound (5e) the formation of N-H-N=C confirmed the formation of the novel compounds. The absorption bands at 3339.2 and 3029.5 cm^{-1} due to N-H stretch, aromatic and aliphatic C-H stretch. The carbonyl stretch was observed at 1652.9 cm^{-1}. The absorption bands appearing at 1539.3, 1514.59 cm^{-1} were due to C=N and C=C. The C-Br and C-F stretch were observed at 576.8 and 1017.5 cm^{-1} respectively. The 100 MHz ^{13}C-NMR recorded for the compound (5e) showed value at δ 18.385 for 1C atom of methyl group, The five carbon atoms of pyridine moiety and 6C atoms of 3-bromo, 4-fluoro phenyl moiety found at δ 157.170, 154.703, 140.796, 131.237, 127.076, 127.044, 121.943, 120.944, 120.758, 118.156, 117.923 ppm. The carbonyl carbon adjacent to pyridine moiety found at δ 161.871 ppm.The 400 MHz ^{1}H-NMR spectrum of compound (5e) showed singlet at δ 2.439 for methyl group attached to C=N moiety. Two doublets appeared at δ 7.566 and 8.778 with a coupling constant *J*=8.8 for four hydrogen atoms of pyridine moiety. The three protons of 3-Br, 4-F-benzene moiety appeared as a multiplet at δ 7.804 and the δ 11.127ppm corresponds to singlet of NH group. The mass spectrum of the compound (4e) molecular ion peak at m/z 336.01 with isotopic peaks at m/z 333.98, consistent with its molecular formula $C_{14}H_{11}BrFN_3O$.

3.1. Biological results

The antibacterial activity of the synthesized target compounds were revealed that these compounds showed to moderate to good activity .out of which the compounds 45e , 45g and 45h showed sensitivity towards both the strains of bacteria used, where as 45c and 45f were found to

show pronounced activity towards gram negative and gram positive bacterial strains respectively. The results are tabulate in table 2.

The average LC-50 values indicate that 5b is more effective followed by 5h, 5d and 5e. Thus, among the test compounds, 5b can be considered as a potential broad spectrum larvicidal agent. If this compound is found to be less toxic to non-target organisms upon other toxicity studies, it may be recommended as a larvicidal agent. Reference standard temephos exhibited very high larvicidal activity. However, it has greater limitations in terms of its toxic effects on non-target organisms.

4. CONCLUSION

The synthesized compounds were screened for the anti-bacterial activity .The compounds 5c,5e, 5g showed promising anti-bacterial activity, especially the compound 5h was found to be highly active for the two strains screened against the reference standard ciprofloxacin and mosquito larvical bioassay was carried out according to standard protocol conducted in accordance with the WHO(World Health Organization,1981).None of the compound met the standard reference but the compounds 5b,5d,5h,5g showed considerably good activity compared to other samples. As revealed from both the screening studies the newly synthesized compounds found to possess good to moderate activity the good activity is attributed due to the presence of substituents like F, OH, Cl and Br in the phenyl ring adjacent N=C moiety .However the presence of substituents like OCH_3 and CH_3 came out with decrease in the biological activity. From the detailed biological analysis the compound 5h revealed to have very good biological activity due to the presence of three chloro groups attached to the phenyl ring.

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