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An efficient synthesis, characterization and antibacterial activity of novel N'-arylidene-4-(2-(piperidin-1-yl) ethoxy) benzohydrazide derivatives Schiff bases

Premlata Gupta, Kamal Jat, Vijendra Singh, Ruchi Shrivastava
(Department of chemistry IPS Academy, Indore, Madhya Pradesh, INDIA)

Abstract: *A series of novel N'-arylidene-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide derivatives* ***(3a-3j)*** *have been synthesized from condensation reaction between 4-(2-(Piperidin-1-yl)ethoxy)benzohydrazide* ***2*** *and various aldehyde. The structures of all these newly synthesized compounds were established on the basis of spectral studies (IR, NMR & MASS) and Element analysis. The anti-microbial activities of these compounds were tested in vitro by agar diffusion cup-plate method against two Gram-negative Escherichia coli, Pseudomonas aeruginosa and two Gram-positive Staphylococcus aureus, Bacillus substilis bacteria and fungi Candida albicans at 100 µg/ml concentration. All these compounds have displayed good or moderate activities against the test microorganisms and all these inactive against C. albicans.*

Keywords*: Antimicrobial activity, hydrazones, piperdine derivatives, Schiff bases.*

I. Introduction

Schiff bases are the most significant class of compounds that has gained much importance in recent years due to their wide range of biological activities and industrial application. Compounds containing the >C=N- (azomethine group) structure are known as Schiff bases, usually synthesized from the condensation of primary amines with active carbonyl groups and they were first reported by Schiff in 1864[1]. These compounds are also known as anils, imines or azomethines. These are found to posses many pharmacological activities such as antimalarial[2], anticancer[3], antibacterial[4], antifungal[5], antitubercular[6], anti-inflammatory[7], antioxidant[8], antiviral[9], anticonvulsant[10], antitumor[11], anti-HIV[12], cytotoxic[13], antidiabetic[14], antihypertensive[15] and analgesic[16] etc. Besides some used as antifertility and enzymatic agents. Several studies showed that the presence of a lone pair of electrons in sp2 hybridized orbital of nitrogen atom of the azomethine group is played an important role in chemical and biological activity. Schiff bases are generally excellent chelating agents[17] especially when a functional group like-OH or –SH is present close to the azomethine group so as to form a five or six member ring with the metal ion. Versatility of Schiff base ligands and biological, analytical and industrial applications of their complexes make further investigations in this area highly desirable.

Based on the higher bio-reactivity of Schiff bases, we have decided to synthesize novel hydrazones Schiff bases containing the piperidine moiety. All the synthesized compounds were characterized by IR, ^{1}H-NMR, Mass spectral, elemental analysis and were screened for their anti-bacterial and anti-fungal activities. The minimum inhibitory concentrations of the compounds were also measured by agar diffusion cup plate method.

II. Experiment

All chemicals and solvents were used of AR-grade. The melting points of all the synthesized compounds were determined in one end open capillary tubes on a Buchi melting point apparatus. IR spectra were recorded on Bruker FT-IR Spectrometer using KBr pellets in the range 4000-600 cm^{-1}. Proton nuclear magnetic resonance (1H-NMR) spectra were recorded on Bruker 400 NMR spectrometer. Chemical shifts are reported in parts per million (ppm) using tetraethyl silane (TMS) as an internal standard. Mass spectra recorded a Waters mass spectrometer. IR, 1H-NMR and MS were consistent with assigned structure. Element analysis (CHN) was undertaken with Thermo Scientific analyzer. The completion of reaction and purity of compounds were checked on thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by ultra violet light 254 nm and developing solvents were chloroform-methanol (9:1).

2.1 Procedure for the preparation of methyl 4-(2-(piperidin-1-yl)ethoxy)benzoate(1)

Methyl 4-(2-(piperidin-1-yl)ethoxy)benzoate was prepared as reported in the literature (Das et al. 2013)

2.2 Procedure for the preparation of 4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (2)

To a dry RBF 15.8g (0.06 mole) methyl 4-(2-(piperidin-1-yl)ethoxy)benzoate (**1)** and 60 ml hydrazine hydrate were heated for 5 hrs at 90-100^{O}C. After completion of reaction (TLC), the reaction mixture cool to

ambient temperature and poured in ice water, filtered, washed with water then recrystallized from absolute ethanol. Product were dried in an oven at 60-65 ^{0}C to give 13.50g 4-(2-(piperidin-1-yl)ethoxy)benzohydrazide. M.P. 98-100^{0}C, yield 85.44%.

RI(cm^{-1}, KBr):-3401.71-3256.14 (NH_2, N-H stretching), 3038.46(Aromatic C-H stretching), 2935.38, 2826.39,2788.72(C-H stretching), 1655.35(C=O stretching), 1604(C-C stretching), 1512(N-H bending), 1342.68(C-O stretching).

MNR(400MHz, DMSO-d_6), δ 9.51 (s, 1H, -NH), 7.22-7.90(m, 4H, Ar), 4.47(s, 2H,-NH_2), 4.21 (t, 2H, OCH_2), 2.71(t, 2H, $OCCH_2$), 2.42(t, 4H, Py), 1.61(m, 2H, Py), 1.49(m, 4H, Py).

MS: m/z- 264.15 (M^+).

2.3 General Procedure for the preparation of Schiff bases(hydrazones) 3a-3j:-

To a dry RBF 1g (0.0038 mole) of 4-(2-(piperidin-1-yl)ethoxy)benzohydrazide**(2)** was refluxed with equal mole of substituted aldehydes in 10 ml absolute ethanol for 5-8 hrs. After completion of reaction (TLC), the reaction mixture cool to ambient temperature and the solid obtained was collected by filtration and recrystallisation in ethanol. Product were dried in an oven at 60-65 ^{0}C to give N'-arylidene-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide **(3a-3j)**

2.3.1 N'-benzylidene-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3a)

IR(cm-^{1}, KBr) :3435.48,3217.07(N-H Stretching),3032.43(Aromatic C-H stretching), 2937.39, 2851.29, 2801.69 (C-H stretching), 1648.04 (C=O),1607(C-C stretching),1563.96 (C=N stretching), 1508.13 (N-H bending), 1368.62 (C-O stretching), 1308.16 (C-N stretching).

^{1}H-NMR (400MHz, DMSO-d_6), δ 11.70 (s, 1H, -NH), 8.46(s, 1H,N=CH), 7.0-8.18(d, 9H, Ar), 4.1(t, 2H, O-CH_2), 2.70(t, 2H, $OCCH_2$), 2.52(t, 4H, Py), 1.52(m, 4H, Py), 1.41(t, 2H, Py).

MS: m/z- 352.16 (M^+).

2.3.2 N'-(4-chlorobenzylidene)-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3b)

IR(KBr): 3431.27,3221.37(N-H Stretching),3042.71(Aromatic C-H stretching), 2934.79, 2861.48, 2810.62 (C-H stretching), 1642.54 (C=O),1612.38(C-C stretching), 1567.40 (C=N stretching), 1511.49 (N-H bending), 1360.73 (C-O stretching), 1301.00 (C-N stretching),715.16(C-Cl stretching),

^{1}H-NMR(400MHz, DMSO-d_6) 11.71 (s, 1H, -NH), 8.45(s, 1H,N=CH), 7.20-8.25(d, 8H, Ar), 4.09(t, 2H, O-CH_2), 2.75(t, 2H, $OCCH_2$), 2.51(t, 4H, Py), 1.54(m, 4H, Py), 1.41(t, 2H, Py).

MS: m/z- 386.12 (M^+).

2.3.3 N'-(4-bromobenzylidene)-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3c)

IR(KBr): 3248.37(N-H Stretching),3052.71(Aromatic C-H stretching), 2951.29, 2846.24, (C-H stretching), 1649.54 (C=O),1614.34(C-C stretching), 1564.41 (C=N stretching), 1515.41 (N-H bending), 1364.41 (C-O stretching), 1308.42 (C-N stretching), 647.25(C-Br stretching).

^{1}H-NMR(400MHz, DMSO-d_6) 11.74 (s, 1H, -NH), 8.42(s, 1H,N=CH), 7.35-8.34(d, 8H, Ar), 4.11(t, 2H, O-CH_2), 2.71(t, 2H, $OCCH_2$),2.54(t, 4H, Py),1.59(m, 4H, Py),1.44(t, 2H, Py)

MS: m/z- 430.16 (M^+)

2.3.4 N'-(4-methylbenzylidene)-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3d)

IR(KBr): 3238.57(N-H Stretching), 3038.32(Aromatic C-H stretching), 2947.31, 2821.54, 2810.60 (C-H stretching), 1651.44 (C=O),1609.23(C-C stretching),1557.76 (C=N stretching), 1528.14 (N-H bending), 1360.52 (C-O stretching), 1321.26 (C-N stretching)

^{1}H-NMR(400MHz, DMSO-d_6)), δ 11.68 (s, 1H, -NH), 8.44(s, 1H,N=CH), 6.91-8.17(d, 8H, Ar), 4.14(t, 2H, O-CH_2), 2.71(t, 2H, $OCCH_2$), 2.46(t, 4H, Py), 2.35(s, 3H, CH_3), 1.54(m, 4H, Py), 1.43(t, 2H, Py).

MS: m/z- 366.19 (M^+)

2.3.5 N'-(4-methoxybenzylidene)-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3e)

IR(KBr): 3227.17(N-H Stretching),3036.40(Aromatic C-H stretching), 2931.24, 2848.27, 2811.64 (C-H stretching), 1645.14 (C=O),1611(C-C stretching),1559.57 (C=N stretching), 1507.43 (N-H bending), 1361.45 (C-O stretching), 1304.11 (C-N stretching)

^{1}H-NMR(400MHz, DMSO-d_6)), δ 11.70 (s, 1H, -NH), 8.44(s, 1H,N=CH), 7.16-8.19(d, 8H, Ar), 4.13(t, 2H, O-CH_2), 3.78(s, 3H, OCH_3) 2.74(t, 2H, $OCCH_2$), 2.52(t, 4H, Py),1.52(m, 4H, Py),1.44(t, 2H, Py),

MS: m/z- 381.16 (M^+)

2.3.6 N'-(4-hydroxybenzylidene)-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3f)

IR(KBr): 3540.28(O-H stretching), 3412.35,3215.24(N-H stretching), 3030.55(Aromatic C-H stretching), 2935.36, 2865.27,2809.29(C-H stretching), 1641.92(C=O stretching), 1607.92(C-C stretching),1568.34(C=N stretching), 1508(N-H bending), 1361.57(C-O stretching), 1302.24(C-N stretching).
^{1}H-NMR(400MHz, DMSO-d_6)), δ 11.74 (s, 1H, -NH), 9.46 (s, 1H, ArOH), 8.48(s, 1H,N=CH), 7.20-8.24(d, 8H, Ar), 4.12(t, 2H, O-CH_2), 2.74(t, 2H, $OCCH_2$),2.50(t, 4H, Py),1.55(m, 4H, Py),1.44(t, 2H, Py).
MS: m/z- 368.20 (M^+)

2.3.7 N'-(4-nitrobenzylidene)-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3g)

IR(KBr): 3276.57 (N-H Stretching), 3117.66 (Aromatic C-H stretching), 2935.42, 2853.96, 2821.67,2879.51 (C-H stretching), 1642.71 (C=O),1607.10 (C-C stretching), 1571.93 (C=N stretching), 1534.99 (N-H bending), 1498.81(Aromatic C-NO_2 stretching), 1333.68 (C-O stretching), 1303.32 (C-N stretching)
^{1}H-NMR(400MHz, DMSO-d_6)), δ 11.(s, 1H, -NH), 8.49(s, 1H,N=CH), 7.16-8.35(d, 8H, Ar), 4.13(t, 2H, O-CH_2), 2.67(t, 2H, $OCCH_2$),2.51(t, 4H, Py),1.51(m, 4H, Py),1.39(t, 2H, Py)
MS: m/z- 397.15 (M^+)

2.3.8 N'-(4-(dimethylamino)benzylidene)-4-(2-(piperidin-1-yl)ethoxy) benzohydrazide (3h*)*

IR(KBr): 3276.57 (N-H Stretching), 3117.66 (Aromatic C-H stretching), 2935.42, 2853.96, 2821.67,2879.51 (C-H stretching), 1642.71 (C=O),1607.10 (C-C stretching), 1571.93 (C=N stretching), 1534.99 (N-H bending), 1333.68 (C-O stretching), 1303.32 (C-N stretching)
^{1}H-NMR(400MHz, DMSO-d_6)), δ 11.72 (s, 1H, -NH), 8.49(s, 1H,N=CH), 7.16-8.35(d, 8H, Ar), 4.13(t, 2H, O-CH_2), 3.11(s, 6H, -$N(CH_3)_2$) 2.67(t, 2H, $OCCH_2$),2.51(t, 4H, Py),1.51(m, 4H, Py),1.39(t, 2H, Py).,
MS m/z 395.22 (M^+)

2.3.9 4-(2-(piperidin-1-yl)ethoxy)-N'-(thiophen-2-ylmethylene)benzohydrazide(3i)

IR(KBr): 3436.65,3246.64 (N-H Stretching), 3058.43 (Aromatic C-H stretching), 2933.19, 2848.41, 2792.62 (C-H stretching), 1644.49 (C=O), 1604.11 (C-C stretching), 1552.95 (C=N stretching), 1506.91 (N-H bending), 1368.97 (C-O stretching), 1299.00 (C-N stretching),706.20(C-S stretching).
^{1}H-NMR(400MHz, DMSO-d_6)), δ 11.68 (s, 1H, -NH), 8.68 (s, 1H,N=CH), 7.02-7.91(d, 7H, Ar), 4.10(t, 2H, O-CH_2), 2.71(t, 2H, $OCCH_2$),2.52(t, 4H, Py),1.53(m, 4H, Py),1.41(t, 2H, Py)
MS: m/z- 358.16 (M^+)

2.3.10N'-(furan-2-ylmethylene)-4-(2-(piperidin-1-yl)ethoxy)benzohydrazide (3j)

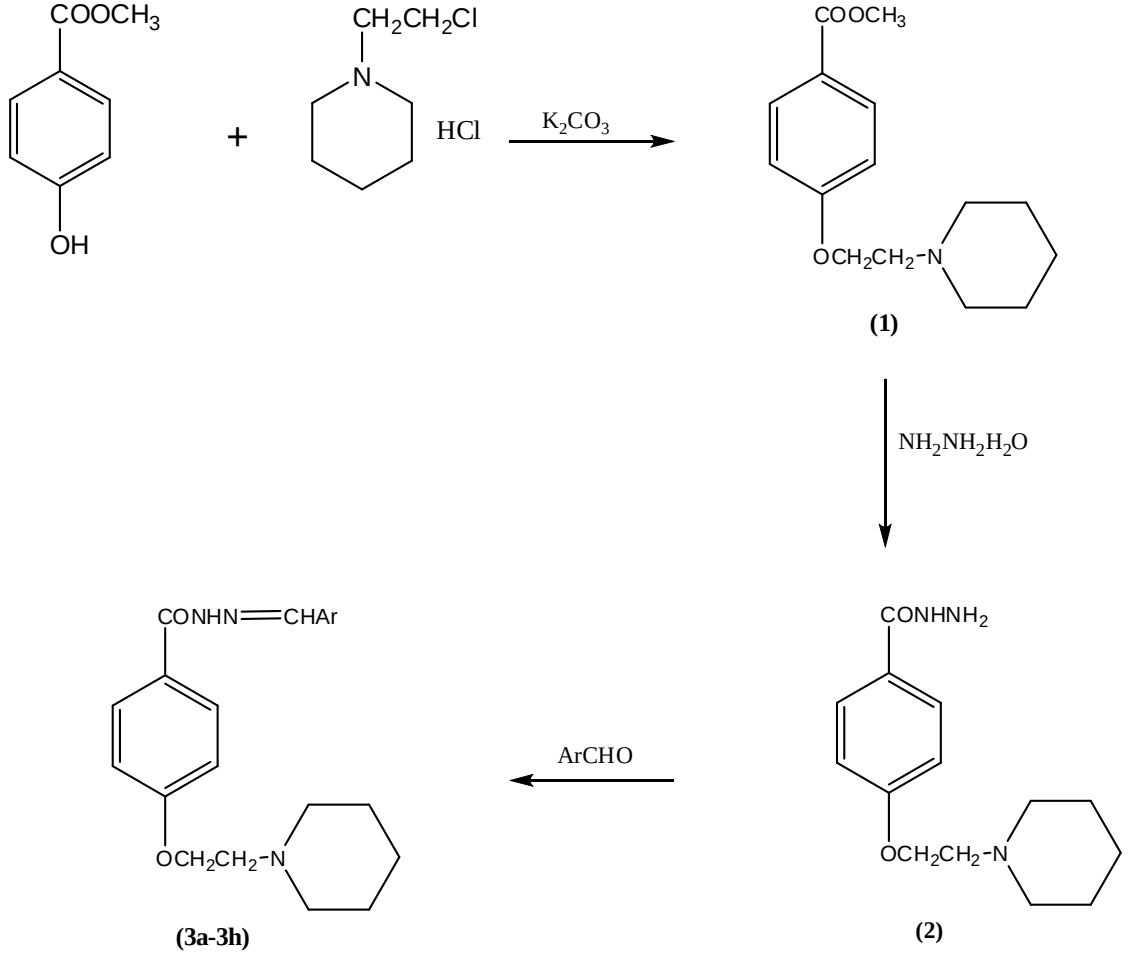
IR(KBr): 3429.29,3225.24 (N-H Stretching), 3044.52 (Aromatic C-H stretching), 2956.24, 2844.25, 2805.39 (C-H stretching), 1648.59 (C=O), 1610.31 (C-C stretching), 1556.97 (C=N stretching), 1504.00 (N-H bending), 1361.20 (C-O stretching), 1304.94 (C-N stretching)
^{1}H-NMR(400MHz, DMSO-d_6)), δ 11.70 (s, 1H, -NH), 8.64(s, 1H,N=CH), 7.07-7.90(d, 7H, Ar), 4.10 (t, 2H, O-CH_2), 2.76(t, 2H, $OCCH_2$), 2.50(t, 4H, Py),1.53(m, 4H, Py),1.42(t, 2H, Py)
MS: m/z- 342.14 (M^+)

III. Results And Discussion

3.1 Chemistry

In the present work, methyl 4-(2-(piperidin-1-yl)ethoxy)benzoate **1** was synthesized by the condensation reaction between methyl 4-hydroxybenzoate and 1-(2-chloroethyl)piperidine in the presence of potassium carbonate in acetone[18]. 4-(2-(Piperidin-1-yl)ethoxy)benzohydrazide 2 were prepared by the reaction of methyl 4-(2-(piperidin-1-yl)ethoxy)benzoate **1** with hydrazine hydrate. This compound **2** was condensed with substituted carbonyl compound in the presence of few drops of acetic acid as a catalytic amount in absolute ethanol to produce Schiff base **(3a-3j).** The synthesis route is shown in scheme 1.

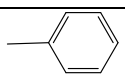
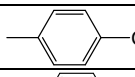
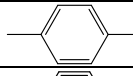
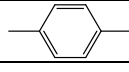
Scheme: - Synthesis routes for piperidine derivatives Schiff bases (3a-3j).

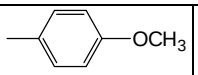
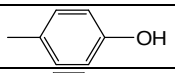
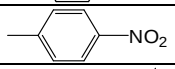
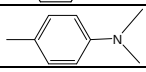
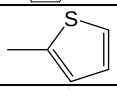
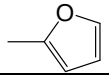


Where Ar is substituted phenyl, furanyl and thiophenyl

The chemical structure of the synthesized Schiff base **(3a-3j)** were characterization by FT-IR, 1H-NMR, MS and elements analysis. The FT-IR spectra of the Schiff bases **(3a-3j)** showed absorption band at 3200-3450 cm^{-1} due to N-H stretching vibration , band at 1640-1655 cm^{-1} due to C=O stretching vibration and band at 1560-1570 cm^{-1} due to azomethine group C=N stretching vibration. The nuclear magnetic resonance (1H-NMR) of Schiff bases (3a-3j) showed singlet at δ 8.40-8.48 indicating presence of azomethine (-N=CH-) proton. The sharp singlet at δ 10.88-11.72 indicating presence of - CONH- proton. In the mass spectrum, Schiff bases **(3a-3j)** showed a peak at m/z, which matches its molecular formula. Physicochemical data and elemental analysis results of the compounds are listed in Table 1. The spectral data of all the compounds are given in Section 2

Table: - (1) Characterization data of compound 3a-3j

Compounds	Ar-	Molecular Formula	Melting Point (^{0}C)	Yield (%)	Elemental Analysis Calc. / (Found)		
					C (%)	H (%)	N (%)
3a		$C_{21}H_{25}N_3O_2$	136-138	82.70	71.77 (71.75)	7.17 (7.20)	11.96 (11.95)
3b		$C_{21}H_{24}ClN_3O_2$	179-181	85.10	65.36 (65.37)	6.27 (6.29)	10.89 (10.88)
3c		$C_{21}H_{24}BrN_3O_2$	202-204	86.45	58.65 (58.61)	5.64 (5.62)	9.75 (9.76)
3d		$C_{22}H_{27}N_3O_2$	186-188	84.35	72.30 (72.33)	7.45 (7.42)	11.50 (11.52)

3e		$C_{22}H_{27}N_3O_3$	190-192	83.56	69.27 (69.26)	7.13 (7.15)	11.02 (11.05)
3f		$C_{21}H_{25}N_3O_3$	186-188	78.24	68.64 (68.67)	6.86 (6.85)	11.44 (11.42)
3g		$C_{21}H_{24}N_4O_4$	175-176	86.17	63.62 (63.60)	6.10 (6.12)	14.13 (14.11)
3h		$C_{23}H_{30}N_4O_2$	212-214	82.95	70.02 (70.00)	7.67 (7.66)	14.20 (14.22)
3i		$C_{19}H_{23}N_3O_2S$	165-168	83.31	63.84 (63.87)	6.49 (6.48)	11.75 (11.73)
3j		$C_{19}H_{23}N_3O_3$	172-174	85.95	66.84 (66.83)	6.79 (6.81)	12.31 (12.29)

3.2 Antimicrobial Activity

The anti-bacterial and antifungal activities of the newly synthesized compounds 3a-3j were evaluated by cup-plate agar diffusion method [19] by measuring the zone of inhibition in mm. All these Novel synthesized compound screened against Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*. And Gram-positive *Staphylococcus aureus, Bacillus substilis* bacteria and fungi *Candida albicans* at 100 µg/ml concentration. The solvent used for the preparation of compound solutions (DMF) did not show inhibition against the tested organisms (negative control). Ciprofloxacin and Fluconazole were used as standard for comparisons antibacterial and antifungal activity. The results of antimicrobial activity of all the newly synthesized compounds were presented in Table 2. Most of the compounds were inactive against C. albicans and all compounds are moderately active but compounds 3g, 3i and 3j are showed good antibacterial activity.

Table:- (2) Antimicrobial activity of newly synthesized compound 3a-3j

Compounds	**Zone of inhibition (in mm)**				
	E. coli	**P. aeruginosa**	**M. luteus**	**B. subtilis**	**C. albicans**
3a	06	08	04	09	-
3b	09	10	07	10	-
3c	08	10	06	08	-
3d	07	08	10	08	-
3e	10	11	09	09	-
3f	15	12	14	17	-
3g	17	19	13	16	-
3h	11	10	08	10	-
3i	22	19	19	21	-
3j	20	21	18	20	-
Ciprofloxacin	25	23	25	24	-
Fluconazole	-	-	-	-	26

IV. Conclusion

We have successfully developed a simple, effective and high yield synthetic procedure for the synthesis of hydrazones Schiff bases **(3a-3j).** The structure of all these compounds have been confirmed by spectroscopic (NMR, IR, MASS) and element analysis data. All the synthesized compounds were evaluated in vitro for their antibacterial activities against two Gram-negative Escherichia coli, Pseudomonas aeruginosa and two Gram-positive Staphylococcus aureus, Bacillus substilis bacteria and fungi Candida albicans at 100 µg/ml concentration. It was observed that the compounds 3f, 3g, 3i, 3j showed good activity whereas rest of the compound displayed moderate activity against bacterial strains and these compounds were found to be inactive against *Candida albicans.*

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