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Research Article

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Devise, Synthesis and Evaluation of Antimicrobial activities of Novel 8-imino Chromone Derivatives by Physicochemical Approach

Jyothi H. Kini*, Vasanta Kumar K. Pai and Yadav D. Bodke

Department of Industrial Chemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shivamogga– 577451, Karnataka, India

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ABSTRACT

*A series of novel 7-Hydroxy-3-methyl-2-phenyl-8(imino) chromone compounds **4(a-p)** were prepared by reacting 8-formyl-7-hydroxy-3-methyl-2-phenyl- chromones with selected amines. The amines were so selected, which facilitate the comprehend survey of the Structure-Activity Relationships. This was accompanied with the reckoning of Lipophilicity (log P), Molar refractive index and molecular weight of the synthesized compounds. All these new compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and Mass spectrometry. The synthesized compounds were screened for in-vitro antimicrobial activities. The Antagonist effects of the different substituent of novel compounds on the antimicrobial activity were discussed. Lead like /drug like qualities of the new compounds was devised by quantitative characterization of the compounds with their computed physicochemical properties.*

Keywords: Chromones, Schiff bases, Antimicrobial, Log P, Lipophilicity.

INTRODUCTION

Chromones (benzo-4-pyrone) and related compounds are widely distributed in nature and have been found to play an important role in a number of biological processes. Chromones have versatile biological activities like anti-cancer shown by Agullo et al.,[1] anti-HIV by Xu et al., [2] antioxidant, anti-inflammatory, antibiotic and also as good vasodilators Middleton et al.[3] and were preferred due to their low mammalian toxicity as per the study by Gabor.[4] Many natural chromones and flavones like Quercetin, Christen, Kaempferol, Myricetin, Apigenin, Luteolin etc., mainly present in apple, tomato, onion etc., found to have the property of inhibiting auto-oxidation reactions and scavenging of free radicals.[5,6] This property delays the decaying of these natural fruits and vegetables. They play a key role in the prevention of cancer. [7-9] They also have “Anti-aging” properties as substantiated by Jones and Hughes.[10] The natural chromones were studied for their SAR (Structure Activity Relationships) [11] and many synthetic chromones are compared to the structure of natural chromones. Those synthetic chromones which are having similar structures, are found to possess similar activities. The automation of the study of the SAR of some natural and synthetic chromones as compiled by Kini et.al [12] assists to choose and design the most biologically potent chromones.

Imino group containing compounds (Schiff bases) are promising owing to their wide range of biological activities and industrial applications.[13] They have been found to exhibit the pharmacological activities such as antimalarial, antitumor, [14] antitubercular, anti-inflammatory, antimicrobial, antiviral, etc. as illustrated by Newman et al. [15] They also serve as a backbone for the synthesis of various metal complexes with heterocyclic ligands.

The interaction between the drug and receptor involves the formation of drug-receptor complex followed by the initiation of the biological effect. The study of the affinity of the ligand for the receptor and the efficacy of the biological activity shows that these are not actually linearly related. But an agonist has to have an optimum affinity to effect maximum biological activity. A drug molecule has to cross both the aqueous and lipid barriers of the biological system to successfully reach the receptor or target site. [16] So it should have both the hydrophilic and hydrophobic nature to reach the receptor site. The lipophilicity and the hydrophilicity of the ligand can be predicted by calculating the partition coefficient P and the *logP* values of the ligand in n-octane and water system. Hansche et.al.[17]The molar refractivity suggests the polarizability of the compounds. The study of which helps to predict the solubility and hence the diffusivity of the molecule into the biological system. The molar mass of the molecule confer the size of the molecule. Since most of the times the molecules with very high molar mass (about 700 dalton) fails passive diffusion into the lipid bilayer.

The present work involves the process of devising the structure of novel compounds with the effective combination of Chromones with functional imino group. Chromones, which are having less mammalian toxicity along with amines, form imino chromones expected to have better activity. The design and development was made by quantitative physicochemical parameter study. We synthesized sixteen new 8-imino-7-hydroxy compounds and investigated for their antimicrobial activity. An attempt was made to synchronize some physicochemical parameters in the path of search for lead molecules.

RESULTS AND DISCUSSION

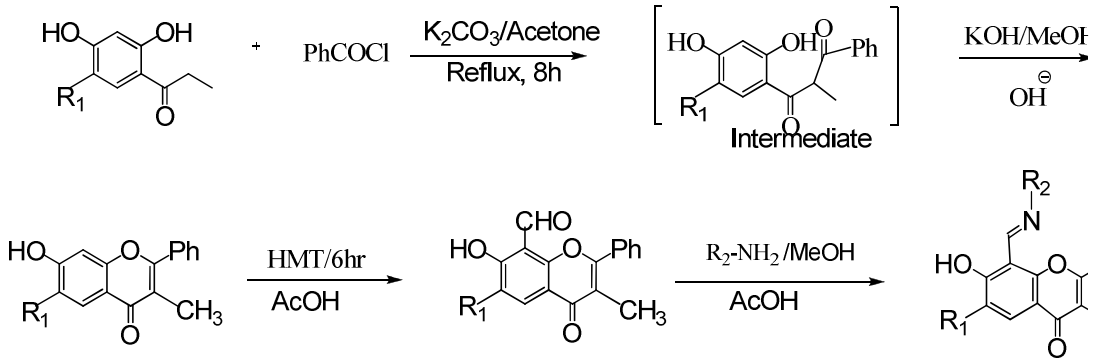
Physicochemical parameters:

Quantitative properties data were shown in Table1. The degree of the antagonistic property of the analogs with the receptor/microbic-cell discloses that the compounds having optimum log P values (4.6-5.1) showed good antimicrobial activities. The molar refractivity of the compounds lying between 10.4-11.6 and the molar mass between 300-490 Dalton were found to be the lead-like molecules. All these quantitative physicochemical parameters hold good with the work of Ghose et.al. [18] By analyzing the quantitative physicochemical parameters, the effective diffusion of the molecules into the biological system could be predicted which was important for any molecule to be treated as lead.

Table 1. Physico chemical parameters of the novel 8-imino compounds.

Compound	Log P	MR(A^3)	Mol. Mass
4a	4.49	106.34	355.39
4b	4.52	111.02	389.83
4c	3.52	116.53	434.28
4d	4.4	111.23	369.41
4e	4.60	113.14	400.38
4f	5.04	117.81	434.83
4g	5.10	120.79	479.28
4h	4.92	118.02	415.42
4i	4.47	111.15	369.41
4j	4.91	115.82	403.86
4k	4.97	118.81	448.31
4l	4.79	116.03	369.41
4m	1.27	93.75	294.30
4n	2.15	86.15	293.30
4o	1.57	96.34	373.20
4p	2.46	91.04	308.33

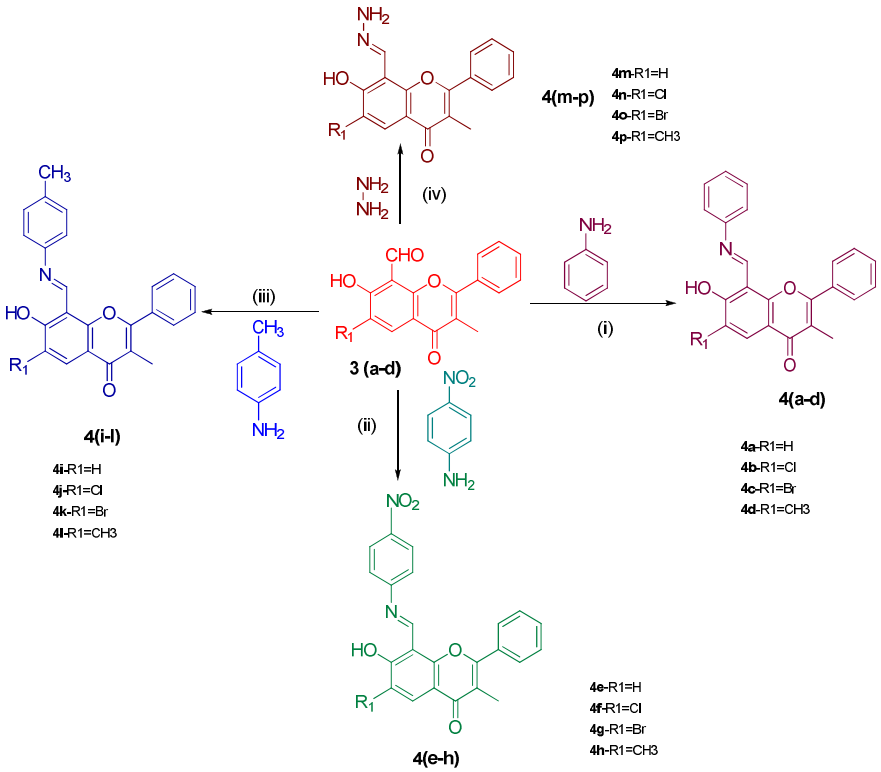
Scheme – 1.



1(a-d), 2 (a-d) and 3(a-d) for R_1 = H (a), Cl (b), Br (c), CH_3 (d)

R_2 = (i) C_6H_5, (ii) $C_6H_4NO_2$(p), (iii) $C_6H_4CH_3$(p), (iv) NH_2

Scheme-2:



Synthesis

8-Formyl-7-hydroxy-3-methyl-2-phenyl chromone **3(a)** was synthesized by the reported method. In the above compound, the hydrogen in the 6th position was replaced with Cl, Br and CH_3 to form compounds **3(b-d)** respectively. This was done to study the effects of these substituent atom/groups on the activity of the analogs. These **3(a-d)** were condensed with a series of primary amines **(i-iv)** by refluxing it in ethanol to form Schiff base compounds **4(a-p)**. The amines were chosen with varied types of substituting groups like non-aromatic (hydrazine hydride), simple aromatic (aniline), with electron withdrawing (p-nitro aniline) and with electron donating (p-toluidine) as shown in (*Scheme1& Schem2*).

The 8-imino compounds formed by the condensation of 8-formyl chromones3(a-d) with four different amines to form 16 new 8-imino chromones (i) -4(a-d), (ii) 4(e-h),(iii) 4(i-l) and (iv) 4(m-p)

The process of synthesis of 8-Formyl compounds **3(a-d)**, possess several steps, which needed skilled workup procedures. The 8-imino compounds synthesized were in moderate to good yields. Further investigations are going on for the easier and greener methods of synthesis of 8-Formyl chromones and their Schiff base compounds.

The structures of synthesized 8-imino analogs were confirmed by ^{1}H-NMR, ^{13}C-NMR and IR spectra. The ^{1}H-NMR spectra of Schiff bases **4(a-p)** showed the absence of a signal around δ 10 for the aldehyde proton, and the appearance of a signal at about δ 9 for HC=N and in the ^{13}C-NMR appearance of a signal at about δ 160 confirmed the desired structures. Furthermore, the IR spectra indicated the appearance of C=N signal near 1620 cm^{-1}.

Antimicrobial activities:

All of these newly synthesized Schiff bases were subsequently evaluated for their biological activities. They were screened for antibacterial activities with gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacterial strains. Also for antifungal activities with *Candida albicans* and *Aspergillus niger* as fungal strains. The *Streptomycin* and *Clotrimazole* were used as positive control for antibacterial and antifungal activities respectively. After the preliminary screening test, the active compounds were tested for their antimicrobial activity against same bacterial and fungal strains with the minimal inhibitory concentrations (MICs) using dimethylsulfoxide (DMSO) as the solvent (negative) control. MICs of the prepared analogs were tested in parallel with the positive and negative control. MICs used for the activity were varying from 12.5µg/mL to 200 µg/mL. The anti-bacterial activity was done by measuring the zone of inhibition using paper disk diffusion method called Kirby-Bauer's method.[21] The method was adopted with some modifications for the prepared compounds. The zone of inhibition was measured in (mm) in each case. The negative control was found to be negligible (<0.002) for both bacterial and fungal strains. The tests were conducted in triplet and the data ere tabulated in Table2 and Table 3.

The antibacterial activity data given in *Table2* were compared with the standard reference *Streptomycin*. Among the synthesized compounds, **4f** showed good antibacterial activity against both bacterial strains. The compounds **4a, 4b, 4e** and **4j** showed good activity against *S.aureus*, whereas **4l** showed good, **4b, 4h** and **4k** possess moderate activity against *E.Coli*.

Table2: Antibacterial activity data of the synthesized compounds

Compound Name	Zone of Inhibition (*mm*)									
	Gram positive: Staphylococcus aureus					*Gram negative: Escherichia coli*				
	0.2mL *	0.1 mL	0.05mL	0.025mL	0.0125 mL	0.2mL	0.1mL	0.05mL	0.025mL	0.0125 mL
4a	19	18	18	16	14	17	14	11	12	11
4b	20	18	17	11	11	21	20	18	18	17
4c	12	11	11	-	-	16	15	13	7	-
4e	20	19	16	15	14	16	14	9	8	6
4f	23	21	19	18	17	25	24	22	20	18
4g	22	22	18	17	15	23	21	21	19	18
4h	16	15	14	12	11	22	20	21	18	16
4j	20	19	18	16	14	17	16	15	8	-
4l	18	18	17	15	14	24	21	20	18	17
Streptomycin	22	20	18	17	16	25	22	22	20	20

*Concentration: 0.2mL = 200µg/mL , - : zone of inhibition < 6*mm*, DMSO(solvent): ~ 0.02*mm*

Similarly for antifungal activities, the data given in *Table3* were compared with the standard reference *Clotrimazole.* The compounds **4g** and **4k** showed comparatively good activity against both antifungal strains. Whereas **4b** and **4j** showed moderately good antifungal activities against *C.albicans* and Compounds **4h** and **4l** possess moderate antifungal activities against *A.niger*.

Table 3 Showing the Antifungal activity data of the synthesized compounds

Compound Name	Zone of Inhibition (in mm)									
	Candida albicans					*Aspergillus niger*				
	0.2mL *	0.1	0.05	0.025	0.0125	0.2	0.1	0.05	0.025	0.0125
4a	14	9	9	6	-	13	11	10	9	-
4b	20	18	16	12	10	14	12	11	9	-
4d	16	15	13	11	10	14	15	12	9	-
4f	23	21	19	16	11	24	20	18	16	11
4g	21	18	16	12	11	22	19	17	16	11
4h	17	15	15	12	10	22	20	18	16	13
4j	20	18	16	12	11	10	8	7	6	-
4l	17	15	14	12	10	20	18	17	16	11
4k	22	18	16	12	11	21	20	18	16	13
Clotrimazole	18	16	15	13	12	20	19	17	16	12

*Concentration: 0.2mL = 200µg/mL , - : zone of inhibition < 6*mm*, DMSO(solvent): ~ 0.02*mm*

From the results of the biological activities, **4f, 4j** containing chlorine and **4g, 4k** having bromine in the 6th position possesses good antibacterial activities. Further, in the compounds **4f** and **4g,** the presence of nitro (electron withdrawing) group may enhance the antagonist property. Compounds **4k**, **4l** containing methyl group showed good to moderate potency against antifungal *C. albicans* and *A. niger*. Also, observations of the activities for imino compounds formed by the condensation of amines with aromatic ring (i-iii) and without aromatic ring (iv) infer that the former possess more activity than the latter. This may be due to the steric stability of the aromatic amines. The scaffold shown in *Fig.1* can be improved accordingly with slight modifications. Further work is now under current investigation to predict the mechanism of action, which remained unclear to date.

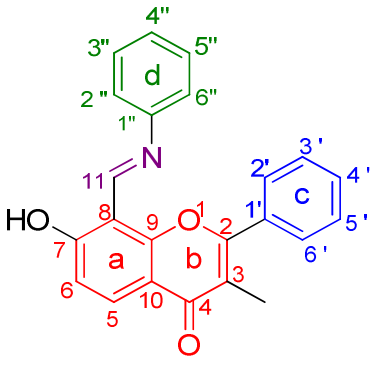


Fig. 1. Showing General structure of 8-imino chromones

Experimental:

Chemistry:

All reagents and solvents used were of AR grade purchased from commercial sources like Sigma-Aldrich, Merck and Himedia. Melting points were determined in open capillary tubes and were uncorrected. Chromatographic purification was done by the column chromatography using Merck silica gel (60 -120meshes). FTIR spectra (KBr) were run on Alpha Brucker-T spectrometer. NMR spectra were recorded on a Bruker-400 MHz spectrometer ^{1}H-NMR 400 MHz, ^{13}C-NMR 100 MHz in $CDCl_3$ solvent using TMS as an internal standard. Chemical shifts (δ) were reported in parts per million (*ppm*) downfield from TMS. Mass spectra were obtained on a Bruker Compass esquire 6000. Elemental analyzes were run on a Thermo Finnigan Flash EA-1112 series. Reactions were monitored by thin-layer chromatography plates coated with 0.2-*mm* silica gel 60 F254 (Merck). TLC plates and were visualized under the UV light.

Preparation of 8-formyl-7-hydroxy -3-methyl-2-phenyl chromones:

The desired Starting material 7-hydroxy-3-methyl-2-phenyl chromone was synthesized by the reaction of respropiophenone **1a** with benzoyl chloride by Baker-Venkataraman rearrangement to afford **2a.** The same procedure was repeated to synthesize **2(b-d)** by employing the starting materials **1(b-d).** Preparation of 8-Formyl-7-hydroxy-3-methyl -2-phenyl chromones **3a** was done by the formylation of **2a** using Hexamethylenetetramine (HMT) by reported method. [19,20] Similarly, the compounds **3b, 3c** and **3d** were formed by using **2b, 2c** and **2d** respectively by employing the same method.

Synthesis of imino chromones of 8-formyl-7-hydroxy chromones: 4(a-p)

General procedure for the synthesis and characterization data of prepared 8-imino (schiff base) compounds 4 (a-p)

8-formyl-7-hydroxy-3-methyl-2-phenyl chromone **3(a)** (10mmol) and Primary amine **(i)** (aniline) (10mmol) and 2-3drops of glacial acetic acid were refluxed in ethanol (20mL) for 3-5 h. The ethanol was evaporated under reduced pressure. Then it was cooled in an ice bath, the solid separated out was the compound **4a** which was then filtered and washed with cold water and dried. The solid was re-crystallized with methanol to afford pure imino compound. The same procedure was repeated with **3(b-d)** with **(i)** to give **4(b-d)**. Similarly, **3(a-d)** was further condensed with other primary amine **(ii)** to form **4(e-h)** and **(iii)** to form **4(i-l)** also with non aromatic amine **(iv)** to form **4(m-p)** as shown in (*Scheme2*). The synthesized compounds were characterized by physical and spectral methods.

7-Hydroxy-3-methyl-2-phenyl-8((phenylimino) methyl)-4H-chromen-4-one (4a):

Yellowish solid, (MeOH), yield 65%, mp 162-164^{o}C;IR (KBr,cm^{-1}) ν_{max} 3014(C-H), 1706(C=O), 1620(C=N),1392(C-O-), 1238(-O-H),1374(C-CH_3)1011, 818(Ar); ^{1}H NMR($CDCl_3$,400MHz,) δ= 5.35 (1H,s,OH), 9.10(1H,s,HC=N), 6.64(1H,d,*J*=8,H-6), 7.12 -7.61 (Ar protons), 8.20(1H,d, *J* =9.2Hz,H-5), 2.43 (3H, s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz,) δ= 184.0(C=O, C-4), 117.4(C, C-10), 113.1(C, C-6), 104.9(C, C-3), 130.9(CH,C-5), 168.1(C-OH, C-7), 160.7(C, C-2), 131.4(CH, C-2′,C-6′), 130.1(CH, C-3′,C-5′), 160.8(C, C=N), 123.3(CH, C 2″, C-6″), 132.2(CH, C-3″,C-5″), 128.2(C, C-4″d ring), 155.1(C-1′c), 116.1(C-C=N, C-1″), 128.1(C-4′c ring), 6.2(CH_3) ; EIMS m/z (%): 354[M]$^{+}$ (8), 101 (100); Anal Calc. for $C_{23}H_{17}NO_3$:C, 77.73;H, 4.82;N,3.94;O,13.51. Found: C, 77.71 ;H, 4.82;N,3.93 ;O,13.50

6-Chloro, 7-hydroxy-3-methyl-2-phenyl-8((phenylimino) methyl)-4H-chromen-4-one (4b):

Pale yellow solid, (MeOH),yield 62%; mp167-168^{o}C; IR(KBr) ν_{max} 3006, 1703, 1620(C=N),1395, 1240, 690, 470(Ar-Cl) cm^{-1}; ^{1}H NMR: ($CDCl_3$,400MHz,) δ= 5.33(1H,s,OH), 9.10(1H,s,HC=N), 7.61(2H,d,*J*=2Hz, H2′,H-6′c), 7.53(2H,dd, *J* =9.6Hz and 1.2Hz, H-3′,H-5′) ,7.31(2H,dd, *J* =8Hz and 2Hz, H-3″,H-5″d), 7.64(2H,d, *J*=4,H-2″,H-6″d ring), 8.10(1H,s,H-5), 7.34(1H,d, *J* =4.8Hz,H-4′c ring), 7.13(1H,d,*J*=2.4Hz,H-4″d ring), 2.45 (3H,s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz) δ= 185.0 (C=O ,C-4), 117.4(C,C-10), 104.9(C, C-3), 121.0(C, C-6), 169.1(C-OH,C-7), 131.5(CH,C-2′,C-6′), 130.8(CH, C-3′,-5′), 161.0(C,C=N), 123.5(CH,C-2″,C-6″), 132.5(CH, C-3″,C-5″), 127.2(CH, C-4″d ring), 116.7(C,C-8), 135.1(CH,C-5), 128.2(CH,C-4′c ring), 6.2(C,CH_3) ; EIMS m/z (%):389 [M]$^{+}$ (11), 391 [M]$^{+2}$, 101 (100); Anal Calc. for $C_{23}H_{16}ClNO_3$:C, 70.86,H, 4.14;Cl,9.09;N,3.59;O,12.31. Found:C,70.67;H, 4.13;Cl,9.0;N,3.57;O,12.30.

6-Bromo, 7-hydroxy-3-methyl-2-phenyl-8((phenylimino) methyl)-4H-chromen-4-one (4c):

Orange solid, (MeOH),yield 65%; mp 180-182^{o}C; IR (KBr) ν_{max} 3010, 1705, 1620,1392, 1239,582,397(Ar-Br) cm^{-1}; ^{1}H NMR: ($CDCl_3$,400MHz,): δ= 5.34(1H,s,OH), 9.11(1H,s, HC=N), 7.61(2H,d,*J*=2Hz, H2′,H-6′c), 7.69(2H,dd, *J* =8Hz and 1.6Hz, ,H-3′,H- 5′), 7.45(2H,dd, *J* =8Hz and 1.2Hz, H-3′,H-5′d), 7.51(2H,d, *J*=4.8,H-2″,H-6″d ring),

8.19(1H,d, *J* =8Hz,H-5), 7.34(1H,d, *J* =1.6Hz,H-4′c ring), 7.18(1H,d, *J* =2Hz,H-4″d ring), 2.43 (3H, CH_3); ^{13}C NMR ($CDCl_3$,100MHz,): δ = 185.2, (C=O,C-4), 117.6(C,C-10), 109.5(C, C-6), 105(C-CH_3,C-3), 169.0 (C-OH,C-7), 131.5(CH,C-2′,C-6′), 131.1(CH, C-3′,-5′), 161.0(C,C=N), 123.5-(CH, C- 2 ″, C-6″), 132.5(CH, C-3″,C-5″), 127.6(CH,C-4″d ring), 118.1(C, C-8), 139.1(CH,C-5), 128.9(CH, C-4′c ring), 6.3(C, CH_3) ; EIMS m/z (%): 435 $[M]^{+2}$, 433 $[M]^+$ (5), 152 (100); Anal Calc. for $C_{23}H_{16}BrNO_3$:C,63.63,H, 3.74;Br,18.4;N,3.24;O,11. Found: C, 63.63;H, 3.72;Br, 18.23; N,3.25;O 10.98.

7-hydroxy-3,6-dimethyl-2-phenyl-8((phenylimino) methyl)-4H-chromen-4-one (4d):
Flaxen white solid, (MeOH), yield 60%; mp 164-166^oC; IR (KBr) ν_{max} 3005,1620,1394,1238, 1374 cm^{-1} ; ^1H NMR ($CDCl_3$,400MHz,): δ= 5.34 (1H,s,OH), 9.10(1H, s, HC=N), 7.56(2H,d,*J*=1.2Hz, H2′,H-6′c), 7.60(2H,dd, *J* =8Hz and 1.2Hz, ,H-3′,H- 5′), 7.46(2H,dd, *J* =10Hz and 2Hz, H-3′,H-5′d), 7.63(2H,d, *J*=4Hz ,H-2″,H-6″d ring), 7.42(1H,d, *J* =12Hz,H-5), 7.14(1H,d, *J* =2Hz,H-4′c ring), 7.07(1H,d, *J* =2Hz,H-4″d ring) , 2.51 (3H,s,H-5-CH_3) 2.45 (3H, s, H-3,CH_3); ^{13}C NMR ($CDCl_3$,100MHz), δ= 183.1 (C=O ,C-4), 117.1(C,C-10), 135.1(CH,C-5), 105.0(C,C-3), 121(C, C-6), 168.8 (C-OH,C-7), 130.8(CH,C-2′,C-6′), 130.9(CH, C-3′,-5′), 160.2(C,C=N), 123.9(CH, C-2″,C-6″),132.8(CH, C-3″,C-5″), 127.9(CH, C-4″d ring), 118.1(C,C-1″), 128.9(CH, C-4′c ring), 18.1(C-$\underline{C}H_3$-C-6), 6.3($\underline{C}H_3$-C-3) ; EIMS m/z (%): 369 $[M]^+$ (7), 81.2(100); Anal Calc. for $C_{24}H_{19}NO_3$:C,78.03,H, 5.15;N,3.78;O,13. Found: C,78.01; H,5.02; N,3.79; O,12.98.

7-Hydroxy-2-phenyl-3-methyl-8-((4-nitrophenylimino)methyl)-4H-chromen-4-one (4e):
Tawny yellow solid (MeOH), yield 66%; mp 184-186^oC; IR (KBr) ν_{max} 3014, 1706, 1620,1392, 1238),1374, 818(Ar), 1550,1302, (Ar-NO_2), 578 cm^{-1}; ^1H NMR: ($CDCl_3$,400MHz,): δ=5.36 (1H,s,OH), 8.99(1H, s,HC=N), 6.91(1H,d,*J*=1.2,H-6), 7.83(2H,d,*J*=1.6Hz, H2′,H-6′c), 7.55(2H,dd, *J* =10 and *J* =1.2Hz, ,H-3′,H- 5′), 8.18(2H,dd, *J* =7.6Hz and 2Hz, H-3′,H-5′d ring), 7.3(2H,d, *J*=10Hz,H-2″,H-6″d ring), 7.8(1H,d, *J* =1.6Hz,H-5), 7.42(1H,d, *J* =1.6Hz,H-4′c ring), 2.53 (3H, s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz,): δ=184.3(C=O,C-4), 116.9(C,C-10), 112.9(C, C-6), 105.0($\underline{C}$-CH_3,C-3), 131(CH, C-5), 168.2(C-OH, C-7), 128.2(CH, C-2′,C-6′), 129.1(CH,C-3′,C-5′), 162.8(C, C=N), 125.1(CH, C-2″,C-6″), 126.2(CH, C-3″,C-5″), 148.5(C-NO_2,C-4″d ring) 116.1(C, C-8), 128.3(CH,C-4′c ring), 6.2(CH_3) ; EIMS m/z (%): 400 $[M]^+$ (6), 101 (100);Anal Calc. for $C_{23}H_{16}N_2O_5$:C, 69.0,H, 4.02;N,7.0;O,19.99. Found: C, 68.99; H, 4.01;N,7.01;O,19.98.

6-Chloro-7-hydroxy-3-methyl-8-((4-nitrophenylimino)methyl)-2-phenyl-4H-chromen-4-one (4f): Amber colored solid,(MeOH), yield 65%; mp189-190^oC ; IR (KBr) ν_{max} 3305,3002, 1704, 1620(C=N),1393, 1240, 690, 470(Ar-Cl), 1550,1301, 793, 578, 445 cm^{-1}; ^1H NMR ($CDCl_3$,400MHz,): δ=5.35(1H,s,OH), 9.10(1H,s,HC=N), 7.84(2H,d,*J*=2Hz, H2′,H-6′c ring) 7.89(2H,dd, *J* =10Hz and 1Hz, ,H-3′,H- 5′), 8.24 (2H,dd, *J* =10Hz and 1.2Hz, H-3″,H-5″d ring), 7.28(2H,d, *J*=4,H-2″,H-6″d ring), 8.10(1H,s,H-5), 7.41(1H,d, *J* =4.8Hz,H-4′c ring), 2.41 (3H,s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz,): δ=184.8 (C=O,C-4), 119.2(C,C-10), 120.8(C, C-6), 104.8(C, C-3), 168.6 (C-OH, C-7), 130.2(CH, C-2′,C-6′), 129.8(CH, C-3′,-5′), 161.3(C, C=N), 123.8(CH, C- 2″, C-6″), 126.2(CH, C-3″,C-5″), 147.7(C, C-4″d ring), 116.7(C, C-8), 155.2(C, C=N), 128.3(CH, C-4′c ring), 6.3 (C-$\underline{C}H_3$ C-3) ; EIMS m/z (%):435 $[M]^{+2}$, 433 $[M]^+$ (4), 131 (100); Anal Calc. for $C_{23}H_{15}ClN_2O_5$:C,63.53,H,3.48;Cl,8.15;N,6.44;O,18.40. Found:C, 63.51;H,3.46;Cl,8.17;N,6.43;O,18.25.

6-Bromo-7-hydroxy-3-methyl-8-((4-nitrophenylimino)methyl)-2-phenyl-4H-chromen-4-one (4g): Brownish yellow solid,(MeOH), yield 60%; mp 192-193^oC ; IR (KBr/cm^{-1}) ν_{max} 3547, 3010, 1705, 1620,1392, 1239,582,397, 1550,1302,374; ^1H NMR ($CDCl_3$,100MHz,): δ= 5.34(1H,s,OH), 9.10(1H,s, HC=N), 7.8(2H,d,*J*=8Hz, H2′,H-6′c), 7.62(2H,dd, *J* =10Hz and 1.6Hz, ,H-3′,H- 5′) , 6.89(2H,dd, *J* =8Hz and 1Hz, H-3′,H-5′d ring), 7.89(2H,d, *J*=4.8,H-2″,H-6″d ring), 8.1(1H,d, *J* =8Hz,H-5), 7.43(1H,d, *J* =1.6Hz,H-4′c ring), 2.43 (3H, s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz,): δ=183.9, (C=O,C-4), 117.6(C,C-10),110.3(C,C-6), 104.9(C,C-3), 155.1(C, C-1″d), 168.8 (C-OH, C-7), 128.5(CH, C-2′,C-6′), 129.2(CH, C-3′,C-5′), 161.1(C=N), 123.6(CH, C -2″,C- 6″)125.5(CH, C-3″,C-5″), 147.6 (C-NO_2,C-4″d ring) 118.4(C, C=N), 139.2.(CH,C-5), 128.9(CH,C-4′c ring), 6.4(CH_3) ; EIMS m/z (%): 478 $[M]^+$(3), 52 (100) 480 $[M]^{+2}$; Anal Calc. For$C_{23}H_{15}BrN_2O_5$:C,57.64;H,3.14;Br,16.60;N,5.84;O,16.69. Found:C,57.61;H,3.10;Br,16.5;N,5.78;O, 16.51.

7-hydroxy-3,6-dimethyl-8-((4-nitrophenylimino)methyl)-2-phenyl-4H-chromen-4-one (4h): Pale yellow solid, (MeOH), yield 64%; mp 165-167^oC ; IR (KBr) ν_{max} 3005,1620,1394,1238, 1374, 1550, 1382 cm^{-1}; ^1H NMR: ($CDCl_3$,400MHz,): δ=5.35(1H,s,OH), 9.21(1H,s,HC=N), 7.80(2H,d,*J*=4Hz, H2′,H-6′c), 7.91(2H,dd, *J* =12Hz and 1.2Hz, ,H-3′,H- 5′), 8.11 (2H,dd, *J* =8Hz and 1.2Hz, H-3″,H-5″d), 7.21(2H,d, *J*=8,H-2″,H-6″d ring), 7.70(1H,s,H-5), 7.44(1H,d, *J* =4.8Hz,H-4′c ring), 2.23 (3H,s,CH_3-H-6), 2.45 (3H,s, CH_3-H-3); ^{13}C NMR ($CDCl_3$,100MHz,):

δ=183.2(C=O,C-4), 116.9(C,C-10), 135.1(CH,C-5), 15.5(CH_3-C-6), 121(C-CH_3, C-6), 168.6 (C-OH, C-7), 129.8(CH,C-2′,C-6′), 128.9(CH, C-3′,C-5′), 160.4(C, C=N), 123.9(CH, C-2″,C-6″), 126.8(CH, C-3″,C-5″), 148.1(C-NO_2, C-4″d ring), 116.1(C, C-1″), 128.2(CH,C-4′c ring), 6.3(CH_3-C-3) ; EIMS m/z (%): 414 $[M]^+$ (5), 85 (100); Anal Calc. for $C_{24}H_{19}N_2O_5$:C,69.56,H, 4.38;N,6.76;O,19.3. Found: C,69.50;H,4.36;N,6.75;O19.1.

7-Hydroxy-3-methyl-2-phenyl-8-((p-tolylimino) methyl)-4H-chromen-4-one (4i):
Orange solid,(MeOH), yield 62%; mp 204-206^{o}C; *IR (KBr)* ν_{max} 3015, 1700(C=O), 1622(C=N),1392, 1238,1374(C-CH_3),1011,818 cm^{-1}; *^1H NMR* ($CDCl_3$,400MHz,): δ=5.36 (1H,s,OH), 8.9(1H,s,HC=N), 6.9(1H,d,*J*=1.2,H-6), 7.8(2H,d,*J*=1.6Hz, H2′,H-6′c ring), 7.5(2H,dd, *J* =10 and *J* =1.2Hz, ,H-3′,H- 5′c ring), 7.4(2H,dd, *J* =6Hz and 0.8Hz, H-3″,H-5″d), 2.43(3H,s,CH_3-4″d ring), 7.3(2H,d, *J*=10Hz,H-2″,H-6″d ring), 7.9(1H,d, *J* =1.2Hz,H-5), 7.4(1H,d, *J* =1Hz,H-4′c ring), 2.53 (3H, s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz,): δ= 184.3 (C=O,C-4), 116.9(C-8), 112.9(C-6), 105.0(CH_3-C-3), 131(CH,C-5), 168.2(C-OH,C-7), 128.2(CH,C-2′,C-6′), 129.1(CH,C-3′,C-5′), 162.8(C=N), 125.1(C-2″,C-6″)126.2(CH,C-3″,C-5″), 137.5(C-CH_3,C-4″d ring) 6.3 (CH_3, C-3) ,116.1(C, C-8), 128.2(CH, C-4′c ring), 6.2 (CH_3) ; EIMS m/z (%): 368 $[M]^+$ (5), 80.5(100); Anal Calc. for $C_{24}H_{19}NO_3$:C, 78.03,H,5.18;N,3.79;O,12.99. Found: C,78.0;H,5.17;N,3.78;O,12.9.

6-Chloro-7-hydroxy-3-methyl-2-phenyl-8-((p-tolylimino)methyl)-4H-chromen-4-one (4j):
Pale brown solid (MeOH), yield 64%; mp 235-237^{o}C; IR (KBr) ν_{max} 3556,3002, 1702, 1621(C=N),1395, 1240, 819, 470 cm^{-1}; ^{1}H NMR ($CDCl_3$,400MHz,): δ= 5.34(1H,s,OH), 9.01(1H, s,HC=N), 7.78(2H,d,*J*=1.6Hz, H2′,H-6′c ring), 8.01(1H, s,H-5), 7.62 (2H,dd, *J* =8Hz and 1.2Hz, ,H-3′,H- 5′), 7.33(2H,dd, *J*=12Hz and 2Hz, H-3″,H-5″d ring), 7.27(2H,d, *J*=10,H-1.2″,H-6″d ring) ,7.44(1H,d, *J* =4.8Hz,H-4′c ring), 2.45(3H,s,CH_3 d ring), 2.54 (3H,s, CH_3); ^{13}C NMR ($CDCl_3$,100MHz,): δ= 184.6 (C=O,C-4), 116.7(C-10), 120.4(C, C-6), 104.8(C,C-3), 167.8(C-OH, C-7), 129.8(CH, C-2′,C-6′), 128.7(CH, C-3′,C-5′), 162.2(C, C=N), 123.4(CH, C-2″,C-6″), 132.4(CH, C-3″,C-5″), 137.2(C-CH_3,C-4″d ring), 21.4(CH_3,C-4″dring), 116.8(C,C-8), 135.0(CH,C-5), 127.4(CH,C-4′c ring), 6.3(CH_3- C-3) ; EIMS m/z (%):404 $[M]^{+2}$,402 $[M]^+$ (4), 84 (100);Anal Calc. for $C_{24}H_{18}ClNO_3$:C,71.38,H,4.49;Cl,8.77;N,3.46;O, 11.88. Found: C, 71.30;H, 4.45;Cl,8.73N,3.45;O,11.86.

6-Bromo-7-hydroxy-3-methyl-2-phenyl-8-((p-tolylimino)methyl)-4H-chromen-4-one (4k):
Brown solid, (MeOH), yield, 55%; mp 241-243^{o}C; IR (KBr) ν_{max} 3298, 3396,(Ar-OH),1450 (Ar), 1620(C=N),1601, 1389,1241, 818,582 cm^{-1}; ^{1}H NMR: ($CDCl_3$,400MHz,): δ= 5.35(1H,s,OH), 8.99(1H, s,HC=N), 7.77(2H,d,*J*=2Hz, H2′,H-6′c), 7.55(2H,dd, *J* =12Hz and 1.2Hz, ,H-3′,H- 5′), 7.45(2H,dd, *J* =10 Hz and 1.2Hz, H-3″,H-5″d ring), 7.43(2H,d, *J*=4.8,H-2″,H-6″d ring), 8.11(1H,d, *J* =6Hz,H-5), 7.54(1H,d, *J* =1.6Hz,H-4′c ring), 2.50(3H,s,CH_3 d ring), 2.43 (3H, s, CH_3-C-3); ^{13}C NMR ($CDCl_3$,100MHz): δ=184.9(C=O, C-4), 116.9(C-10), 110.2(C-6), 104.9(C-3), 168.2 (C-OH, C-7), 129.9(CH, C-2′,C-6′), 128.9(CH, C-3′,C-5′c), 161.4(C, C=N), 124.1(CH, C-2″,C-6″), 132.6 (CH, C-3″,C-5″), 137.5(C-CH_3, C-4″d ring), 138.7(CH, C-5), 128.4(CH, C-4′c ring), 21.8(CH_3-C-4″d ring), 6.4(CH_3-C-3) ; EIMS m/z (%): 449 $[M]^{+2}$, 447$[M]^+$(6),81.2(100);AnalCalc.for$C_{24}H_{18}BrNO_3$: C,64.30, H,4.05;Br,17.82;N,3.12;O,10.71.Found: ,64.29;H,4.01;N,3.12;O,10.70.

7-hydroxy-3,6-dimethyl-2-phenyl-8-((p-tolylimino)methyl)-4H-chromen-4-one (4l):
Grey solid, (MeOH), yield, 67%; mp 237-238^{o}C; IR (KBr) ν_{max} 3005,1620,1394,1238, 1374, 818, 584 cm^{-1}; ^{1}H NMR: ($CDCl_3$,400MHz,) :δ=5.35(1H,s,OH), 9.10(1H,s, HC=N), 7.87(2H,d,*J*=4Hz, H2′,H-6′c), 7.55(2H,dd, *J* =8Hz and 1.2Hz, H-3′,H- 5′), 7.45 (2H,dd, *J* =10Hz and 1.2Hz, H-3″,H-5″d ring), 7.83 (2H,d, *J*=6,H-2″,H-6″d ring), 2.45(3H,s,CH_3 d ring), 7.41(1H,d, *J* =8Hz,H-4′c ring), 2.23 (3H,s,CH_3-C-6), 2.40 (3H, s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz): δ=183.9(C=O,C-4), 160.7(C,C-2), 116.5(C,C-10), 135.2(CH, C-5), 104.5(C,C-3), 121.2(C,C-6), 168.3 (C-OH, C-7), 134.1(C-1′), 129.7(CH, C-2′,C-6′), 128.8(CH, C-3′,C-5′), 160.4(C, C=N), 123.7(CH, C-2″,C-6″), 130.7 (CH,C-3″,C-5″), 138.0(C-CH_3,C-4″d ring), 117.9(C-1″), 127.9(CH,C-4′c ring), 21.8(C-$CH_3$4″d ring), 15.7(CH_3-C-6), 6.2(CH_3-C-3); EIMS m/z(%) 368$[M]^+$ (4), 64(100); Anal Calc. for $C_{24}H_{19}NO_3$:C,78.31, H,5.52;N,3.650;O,12.52. Found: C,78.25;H,5.52;N,3.64;O12.51.

7-Hydroxy-3-methyl-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (4m):
Buff colored solid, (MeOH); yield 55%; mp 172-173^{o}C; IR (KBr) ν_{max} 3405,3304(-NH_2) 3015, 1700(C=O), 1622(C=N),1392, 1238,1374(C-CH_3),1011,818 cm^{-1}; ^{1}H NMR ($CDCl_3$,400MHz,): δ= 5.34 (1H,s,OH), 8.88(1H,s, HC=N), 6.74(1H,d,*J*=8,H-6), 7.84(2H,d,*J*=2Hz, H2′,H-6′c ring), 7.55(2H,dd, *J* =8 and *J* =1.6Hz, ,H-3′,H- 5′), 7.89(1H,d, *J*=8,HC-5), 7.31(1H,d, *J* =16.4Hz,H-4′c ring), 2.20(2H, s,H_2N), 2.43 (3H, s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz): δ=184.1 (C=O,C-4), 161.0(C, C-2), 117.3(C, C-10), 113.1(CH, C-6), 104.9(C-C-3), 167.7(C-OH, C-7), 128.1(CH,C-2′,C-6′), 129.2(CH,C-3′,C-5′), 159.8(C, C=N), 116.1(C-C=N,-1″d ring), 131.1(CH, C-5),

128.1(CH,C-4′c ring), 6.2 (CH_3-C-3) ; EIMS m/z (%): 293$[M]^+$ (8), 50(100); Anal Calc. for $C_{17}H_{14}N_2O_3$:C, 69.38,H, 4.79;N,9.52;O,16.31. Found: C, 69.35;H,4.78;N,9.52;O, 16.30.

6-Chloro-7-hydroxy-3-methyl-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (4n):
Ash colored solid,(MeOH); yield 54%; mp 152-154^{o}C; IR (KBr) ν_{max} 3405,3304, 3556,3002, 1702, 1621(C=N),1395, 1240, 819, 470 cm^{-1};^{1}H NMR: ($CDCl_3$,400MHz,): δ =5.33 (1H,s,OH), 8.92(1H, s,HC=N), 7.87(2H,d,*J*=2Hz, H2′,H-6′c), 7.54(2H,dd, *J* =10 and *J* =1.6Hz, ,H-3′,H- 5′) 8.1(1H, s,HC-5), 7.35(1H,d, *J* =12Hz,H-4′c ring), 2.34(2H,s, H_2N), 2.41 (3H, s,CH_3); ^{13}C NMR ($CDCl_3$,100MHz): δ = 183.9 (C=O,C-4), 118.3(C, C-10), 160.7(C, C-2), 120.2(C, C-6), 104.9(C, C-3), 135.1(CH, C-5), 167.7(C-OH, C-7), 128.1(CH,C-2′,C-6′), 129.2(CH,C-3′,-5′), 163.0(C=N), 116.8(C-C=N-1″d ring), 154.9(C-C=N, C-8),128.1(CH, C-4′c ring), 6.2 (CH_3, C-3) ; EIMS m/z (%):329$[M]^{+2}$, 327$[M]^+$ (12), 68(100); Anal Calc. for $C_{17}H_{13}ClN_2O_3$:C, 62.11,H,3.99;Cl,10.78,N,8.52; O,14.60. Found: C, 62.10;H, 3.98;N,8.50;O, 14.60.

6-Bromo-7-hydroxy-3-methyl-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (4o):
Pale brown solid,(MeOH); yield 52%; mp 160-162^{o}C; IR (KBr) ν_{max} 3547,3010, 1705, 1620,1392, 1239,582,397 cm^{-1}; ^{1}H NMR: ($CDCl_3$,400MHz,):δ=5.35(1H,s,OH), 8.63(1H,s,HC=N),7.88(2H,d,*J*=1.6Hz, H2′,H-6′c), 7.54(2H,dd, *J* =9Hz and *J* =1.2Hz, ,H-3′,H- 5′), 8.01(1H,s, HC-5) 7.30(1H,d, *J* =2Hz,H-4′c ring), 2.22(2H,s, H_2N), 2.43 (3H,s, CH_3); ^{13}C NMR ($CDCl_3$,100MHz): δ =183.6 (C=O,C-4), 118.2(C, C-10), 160.8(C, C-2), 110.0(C, C-6), 104.8(C, C-3), 138.2(CH, C-5), 168.2(C-OH, C-7), 128.2(CH, C-2′,C-6′), 129.4(CH, C-3′,-5′), 159.1(C, C=N), 117.2(C, C-8), 128.2(CH, C-4′c ring), 6.2 (CH_3, C-3) ; EIMS m/z (%):374$[M]^{+2}$, 372$[M]^+$ (7), 78.5(100);Anal Calc. for $C_{17}H_{13}BrN_2O_3$:C, 54.72,H,3.50;Br,21.41,N,7.51;O,12.86. Found: C, 54.70,H, 3.48;Br, 21.40;N, 7.52;O, 12.86.

7-hydroxy-3,6-dimethyl-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (4p):
Off white solid,(MeOH); yield 44%; mp181-183^{o}C; IR (KBr) ν_{max} 3405,3304, 3005,1620,1394,1238, 1374, 818 cm^{-1}; ^{1}H NMR: ($CDCl_3$,400MHz,): δ = 5.34(1H,s,OH), 8.76(1H,s, HC=N), 7.78(2H,d,*J*=6Hz, H2′,H-6′c), 7.54(2H,dd, *J* =10Hz and 1.2Hz, ,H-3′,H- 5′), 7.48(1H,d, *J* =8Hz,H-4′c ring), 2.21 (3H,s,CH_3-H-6), 2.44 (3H,s,CH_3-C-3); ^{13}C NMR ($CDCl_3$,100MHz): δ = 183.9(C=O, C-4), 116.6(C, C-10), 135.3(CH, C-5), 161.1(C, C-2), 104.4(C, C-3), 121.3 (C, C-6), 168.4 (C-OH, C-7), 129.6(CH, C-2′,C-6′), 128.7(CH, C-3′,C-5′), 158.4(C, C=N-NH_2), 117.5(C-C=N), 127.9(CH,C-4′c ring), 15.8(CH_3, C-6), 6.3(CH_3,C-3); EIMS m/z (%): 307$[M]^{+1}$ (5), 88(100);Anal Calc. for $C_{18}H_{16}N_2O_3$:C,70.12,H,5.23;N,9.09;O,15.57. Found: C,70.11;H,5.22;N,9.10;O15.56.

Antimicrobial activities:
The Antimicrobial screening of the new compounds was done as mentioned in results and discussion section according to the standard procedure. For antibacterial activities, the media were prepared with beef extract, peptone and agar and the pH was adjusted to 5.8-6.5. For screening of anti-fungal activity, the media were prepared using potato, sucrose and agar. The solvent control used was DMSO for both and the activity was compared with standard references, *Streptomycin* for bacterial and *Clotrimazole* for fungal strains. The procedure was as given in ASM Microbe Library. [21]The inhibition zones caused by the various compounds on the microorganisms were examined. After the preliminary screening test, the active compounds were tested for their antimicrobial activity against same bacteria and fungi strains as that of preliminary tests. The minimal inhibitory concentrations (MICs) of the tested compounds were determined by the dilution method with series of concentrations of the compounds which varied as 200µg/mL, 100µg/mL, 50µg/mL, 25µg/mL and 12.5µg/mL(i.e. 0.2mL, 0.1mL, 0.05mL, 0.025mL and 0.0125mL respectively). The zone of inhibition was measured in (*mm)* for each type of bacterial strains and also for the reference. [22] The results were as in *Table1*.

A similar method was adopted for screening anti-fungal activities with same varying concentrations and with same two fungal strains. The zone of inhibition was recorded in *mm* in each case, including the reference. The data were as in *Table2*.

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Conflict of Interest:
The authors declare that they have no conflict of interest.

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