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Research Article

Physico-Chemical Approach in Design, Synthesis and Antimicrobial Study of some Novel Schiff bases of Chromones

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ABSTRACT

A series of novel Schiff base derivatives of chromones 5(a-p) were prepared by reacting 8-formyl-7-hydroxy-2-phenyl-chromones with selected amines. The microwave assisted synthesis of 8-formyl-7-hydroxy-2-phenyl-chromones contributes towards the green chemistry by reducing the heating time. This was accompanied with the physicochemical parameters like Lipophilicity (CLogP), Molar refractive index (CMR), topological surface area (tPSA) and the molecular weight of the synthesized compounds. The Antagonist effects of the different substituent of novel compounds on the microbial activity were discussed. The novel compounds were designed with Lead like qualities by computing their quantitative physicochemical parameters.

Keywords: Chromones, Schiff bases, Antimicrobial, LogP, Physicochemical parameter and Microwave synthesis.

1. INTRODUCTION

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 to the receptor and the efficacy of the biological activity imparts important aspects of chemical biology. An agonist must possess an optimum affinity to effect maximum biological activity. A drug molecule has to cross both the aqueous and lipid barriers of the cell to successfully reach the receptor or target site.[1] So it should have both the hydrophilic and lipophilic nature to reach the receptor site. The study by Hansche et.al. [2], on the partition coefficient of the ligand in n-octane/water system, gives an idea about the diffusion of the ligand into the cell membrane. Apart from the partition coefficient, we have also studied the other aspects of the bio-diffusion and bio-availability like the molecular refractivity studies, the size of the molecule is also important as the large molecules i.e. the molecules with molecular weight > 600 usually finds difficulty in crossing the cell membrane as illustrated by Lipinski, s rule of 5.[3] The topological surface area of the molecule accounts for the toxicity of the molecule. Thus the physicochemical approach in designing avoids the synthesis of the molecules with low diffusivity and high tPSA.

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.,[4] antioxidant, anti-inflammatory, antibiotic, anti-HIV by Xu et al.,[5] and also as good vasodilators Middleton et al[6].and were preferred due to their low mammalian toxicity as per the study by Gabor, in 1991.[7] The natural chromones were studied for their SAR (Structure Activity Relationships) by S.Kumar and A.K. Pandey[8] and the computer assisted designing of many synthetic chromones having the similar structures to that of natural chromones, are found to possess similar activities.

Schiff base compound are having promising wide range of biological activities and industrial applications Wadher et. al.[9] They have been found to have the

pharmacological activities such as antimalarial, antitumor, Hu et. al.,[10] antitubercular, anti-inflammatory, antimicrobial and antiviral, etc. as illustrated by Newman et al.[11] They also serve as a backbone for the synthesis of various metal complexes with heterocyclic ligands. We have devised the structure of new Schiff base derivatives of 7-hydroxy chromones using automation method as compiled by Kini et.al,[12] and investigated their potent biological activity.

Green synthesis and a healthy environment are now on the frontier of all chemical industries. The present work involves physicochemical approach in designing of the novel compounds with the effective combination of Chromones which are having less mammalian toxicity with functional imino group. Currently, the Microwave assisted synthesis is gaining importance. Since the time required is less compared to the conventional method of heating and the solvents required were less and non-hazardous and the purity of the compound is also high as there are fewer issues of separation and purification. An attempt was made to incorporate some green synthesis to have a healthy environment.

2. MATERIALS AND METHODS

All reagents and solvents used were of AR grade purchased from commercial sources (Sigma-Aldrich, Merck and Himedia) and used without further purification. ONIDA Power solo 20 digital domestic Microwave oven was used for the synthesis with high MW compatible glasswares like tubes and conical flasks. 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 a $CDCl_3$ 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 analyses were run on a Thermo Finnigan Flash EA-1112 series. Reactions were monitored by thin-layer chromatography plates coated with 0.2mm silica gel 60 F254 (Merck). TLC plates were visualized under the UV light.

2.1Preparation of 8-formyl-7-hydroxy -2-phenyl chromones:

The desired Starting material i.e. the derivatives of 8-Formyl-7-hydroxy-2-phenyl-3-methyl chromones, 6-Chloro, 8-formyl-7-hydroxy-2-phenyl chromones, 6-Bromo 8-Formyl-7-hydroxy-2-phenyl chromones, 8-Formyl-7-hydroxy-6-methyl-2-phenyl, chromones were synthesized using Microwave irradiation method.[13] The solvents used were Chlorobenzene and ethanol. The MW power (80%) was irradiated for 5-8 mins. The resulting compounds were characterized by IR, 1H NMR and Mass spectrometry. The Microwave acts as the greener way compared to the conventional heating method. The synthesis was done using Microwave and the products obtained were >95% pure. The synthetic scheme was as in Scheme 1.

2.2 Synthesis of Schiff bases of chromones of 8-formyl-7-hydroxy chromones: 5(a-p)

2.2.1 General procedure for the synthesis and characterization data of prepared Schiff base compounds 5 (a-p)

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

2.2.1a:7-Hydroxy-2-phenyl-8((phenylimino) methyl)-4H-chromen-4-one (5a):

Orange solid, (MeOH), yield 73%, mp 152-154^{o}C;IR (KBr,cm^{-1}) ν_{max} 3014(C-H), 1706(C=O), 1620(C=N),1392(C-O-), 1238(-O-H), 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); ^{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) ; EIMS m/z (%): 354[M]$^+$ (8), 101 (100); Anal Calc. for $C_{22}H_{15}NO_3$:C, 77.73;H, 4.42;N,4.04;O,14.51. Found: C,77.71 ;H, 4.41 ;N,4.03 ;O14.49 .

2.2.1b:6-Chloro-7-hydroxy-2-phenyl-8((phenylimino)methyl)-4H-chromen-4-one (5b): Yellow solid, (MeOH),yield 78%; mp164-165^{o}C;

IR(KBr) ν_{max} 3006, 1703, 1620(C=N), 1240, 690, 470(Ar-Cl) cm^{-1}; ^1H 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); ^{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) ; EIMS m/z (%):389 $[M]^+$ (11), 391 $[M]^{+2}$, 101 (100); Anal Calc. for $C_{22}H_{14}ClNO_3$: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;O12.30.

2.2.1c:6-Bromo-7-hydroxy-2-phenyl-8-((phenylimino) methyl)-4H-chromen-4-one (5c): Brown solid, (MeOH),yield 65%; mp 170-172°C; IR (KBr) ν_{max} 3010, 1705, 1620, 1239,582,397(Ar-Br) cm^{-1}; ^1H 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); ^{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-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); EIMS m/z (%): 422.0 $[M]^{+2}$, 420.0 $[M]^+$ (5), 152 (100); Anal Calc. for $C_{22}H_{14}BrNO_3$:C,63.63,H, 3.74;Br,18.24;N,3.24;O,11. Found: C, 63.63;H, 3.72;Br, 18.23; N,3.25;O 10.98.

2.2.1d:6-Methyl-7-hydroxy-2-phenyl-8-((phenylimino) methyl)-4H-chromen-4-one (5d): Orange red solid, (MeOH), yield 70%; mp 164-166°C; IR (KBr) ν_{max} 3005,1620, 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) ; ^{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); EIMS m/z (%): 355.0 $[M]^+$ (7), 78.2(100); Anal Calc. for $C_{23}H_{17}NO_3$:C,78.03,H, 5.15;N,3.78;O,13. Found: C,78.01; H,5.02; N,3.79; O,12.98.

2.2.1e:7-Hydroxy-2-phenyl-8-((4-nitrophenylimino)methyl)-4H-chromen-4-one (5e): Pale yellowsolid (MeOH), yield 68%; mp 174-176°C; IR (KBr) ν_{max} 3014, 1706, 1620, 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); ^{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(C, 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); EIMS m/z (%): 386.1 $[M]^+$+H (6), 98.2 (100);Anal Calc. for $C_{22}H_{14}N_2O_5$:C, 69.0,H, 3.72;N,7.3;O,20.9. Found: C, 68.99; H, 3.71;N,7.1;O,20.80.

2.2.1f:6-Chloro-7-hydroxy-8-((4-nitrophenylimino)methyl)-2-phenyl-4H-chromen-4-one (5f): Brownish colored solid,(MeOH), yield 64%; mp169-170°C ; IR (KBr) ν_{max} 3305,3002, 1704, 1620(C=N), 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); ^{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); EIMS m/z (%):420.05 $[M]^+$+H, 421.1 $[M]^+$+2 (4), 129 (100); Anal Calc. for $C_{22}H_{13}ClN_2O_5$:C,63.53,H,3.48;Cl,8.15;N,6.44;O,18.40. Found:C, 63.53;H,3.46;Cl,8.17;N,6.43;O,18.35.

2.2.1:.6-Bromo-7-hydroxy-8-((4-nitrophenylimino)methyl)-2-phenyl-4H-chromen-4-one (5g): Brownish yellow solid,(MeOH), yield 75%; mp 182-183°C ; IR (KBr/cm^{-1}) ν_{max} 3547, 3010, 1705, 1620, 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; ^{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); EIMS m/z (%): 464.0 $[M]^+$+H (3), 52 (100) 465.1$[M]^{+2}$; Anal Calc. For $C_{22}H_{13}BrN_2O_5$:C,56.79;H,2.82;Br,17.17;N,6.01;O,17.19.Found:C,57.79;H,2.80;Br,17.09;N,6.0;O, 17.18.

2.2.1h6-Methyl-7-hydroxy-8-((4-nitrophenylimino)methyl)-2-phenyl-4H-chromen-4-one (5h): Brownish red solid, (MeOH), yield 62%; mp 165-167^{o}C ; IR (KBr) ν_{max} 3005,1620,1394,1238, 1374, 1550, 1382 cm^{-1}; ^{1}H 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); ^{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); EIMS m/z (%): 400.12 $[M]^+$+H, (5), 401.1 $[M]^+$, 85 (100); Anal Calc. for $C_{23}H_{16}N_2O_5$:C,69.05,H, 4.03;N,6.98, O,19.99. Found: C,69.05;H,4.04;N,6.97;O19.98.

2.2.1i:7-Hydroxy-2-phenyl-8-((p-tolylimino) methyl)-4H-chromen-4-one (5i):
Orange red solid,(MeOH), yield 69%; mp 192-194^{o}C; *IR (KBr)* ν_{max} 3015, 1700(C=O), 1622(C=N), 1238,1374(C-CH_3),1011,818 cm^{-1}; *^{1}H 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); ^{13}C NMR ($CDCl_3$,100MHz,): *δ*= 184.3 (C=O,C-4), 116.9(C-8), 112.9(C-6), 105.0(C, 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),116.1(C, C-8), 128.2(CH, C-4′c ring), 6.2 (CH_3, d ring) ; EIMS m/z (%): 355.13 $[M]^+$+H (5), 78.5(100); Anal Calc. for $C_{23}H_{17}NO_3$:C, 77.80,H,4.81;N,3.97;O,13.51. Found: C,77.80;H,4.87;N,3.98;O,13.50.

2.2.1j:6-Chloro-7-hydroxy-2-phenyl-8-((p-tolylimino)methyl)-4H-chromen-4-one (5j):
Pale brown solid (MeOH), yield 64%; mp 181-182^{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); ^{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); EIMS m/z (%):389.08 $[M]^+$+H,390.02 $[M]^+$+2 (4), 84 (100);Anal Calc. for $C_{23}H_{16}ClNO_3$:C,70.83,H,4.14;Cl,9.07;N,3.59;O,12.31. Found: C, 70.80;H, 4.15;Cl,9.03N,3.58;O,12.31.

2.2.1k:6-Bromo-7-hydroxy-2-phenyl-8-((p-tolylimino)methyl)-4H-chromen-4-one (5k):
Brown solid, (MeOH), yield, 65%; mp 197-198^{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); ^{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(C-H_3-C-4″d ring); EIMS m/z (%): 433.03 $[M]^+$+H, 435.05 $[M]^+$+2 (6), 78.2(100); Anal Calc. for $C_{23}H_{16}BrNO_3$:C,63.61,H, 3.75;Br,18.42;N,3.22;O,11.07. Found: C,63.62;H,3.70;N,3.21;O,11.05.

2.2.1l:6-Methyl-7-hydroxy-2-phenyl-8-((p-tolylimino)methyl)-4H-chromen-4-one (5l):
Orange solid, (MeOH), yield, 77%; mp 137-138^{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); ^{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$4″d ring), 15.7(CH_3-C-6); EIMS m/z (%): 369.15$[M]^+$+H (4), 64(100); Anal Calc. for $C_{24}H_{19}NO_3$:C,78.03,H,5.19;N,3.76;O,12.98. Found: C,78.02;H,5.17;N,3.66;O12.98.

2.2.1m:7-Hydroxy-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (5m):

Grey colored solid, (MeOH); yield 65%; mp 178-179^{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); EIMS m/z (%): 280.08$[M]^+$ +H(8), 50(100); Anal Calc. for $C_{16}H_{12}N_2O_3$:C, 68.53,H, 4.32;N,9.98;O,17.14. Found: C, 68.53;H,4.32;N,9.97;O, 17.12.

2.2.1n:6-Chloro-7-hydroxy-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (5n):

Brown colored solid,(MeOH); yield 74%; mp 142-144^{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); EIMS m/z (%):314.73$[M]^+$+H, 315.8$[M]^+$+2 (12), 68(100); Anal Calc. for $C_{16}H_{11}ClN_2O_3$:C, 61.11,H,3.49;Cl,11.21,N,8.92;O,15.26. Found: C, 61.10;H, 3.48;N,8.90;O, 15.26.

2.2.1o.6-Bromo-7-hydroxy-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (5o):

Ash brown solid,(MeOH); yield 72%; mp 160-161^{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); EIMS m/z (%):358.04$[M]^+$+H, 360.2$[M]^+$+2 (7), 76.8(100);Anal Calc. for $C_{16}H_{11}BrN_2O_3$:C, 53.57,H,3.09;Br,22.24,N,7.80;O,13.36. Found: C, 53.57,H, 3.08;Br, 22.24;N, 7.8;O, 13.36.

2.2.1p:6-Methyl-7-hydroxy-2-phenyl-8-(hydrazonomethyl)-4H-chromen-4-one (5p):

Ash colored solid,(MeOH); yield 84%; 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); EIMS m/z (%): 294.10$[M]^+$+H(5), 88(100);Anal Calc. for $C_{17}H_{14}N_2O_3$:C,69.38,H,4.78;N,9.53;O,16.31. Found: C,69.37; H,4.78;N,9.51;O16.30.

2.3 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 MicrobeLibrary.[14] 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 were measured in *mm* for each type of bacterial strains and also for the reference. The activity data were as in ***Table2***.

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 ***Table3***.

3. RESULTS AND DISCUSSION

3.1 Physicochemical parameters:

Quantitative physicochemical properties data were shown in ***Table1*****.** The CLogP value of the compound is the measure of their lipophilicity that helps them to enter into the cell. The degree of an antagonistic property of the analogs with the receptor/microbic protein reveals that the compounds having optimum CLogP values (3-5.1) showed good antimicrobial activities. The molecular refractivity (CMR) lying between (9-12) are expected to be lead like molecules. Also, the molecular mass lies between 290-490daltons.[15,16] This holds good with the work of Ghose et.al.[17] Further, for the compounds having CLogP greater than 3 and calculate topological surface area of the compounds (tPSA) less than $75A^{o2}$ are six times more likely to elicit in vivo effects at concentrations below 10µM of total drug compared to the compounds that have CLogP >3 and tPSA> $75A^{o2}$ are more likely to have increased propensity for off-target pharmacology.[18]

3.2 Synthesis:

8-Formyl-7-hydroxy-3-methyl-2-phenyl chromone **4(a)** was synthesized by the reported method. In the above compound, the hydrogen in the 6^{th} position was replaced with Cl, Br and CH_3 to form compounds **4(b-d)** respectively. This was done to study the effects of these substituent atom/groups on the activity of the analogs ***(Scheme 1)***. These **4(a-d)** were condensed with a series of amines **(i-iv)** by refluxing it in ethanol to form Schiff base compounds **5(a-p)**. The amines were chosen with varied types of substituting groups like non-aromatic (NH_2-NH_2), (hydrazine hydride), simple aromatic (aniline), with electron withdrawing (NO_2), (p-nitro aniline) and with electron donating (CH_3) group (p-toluidine) as shown in **(*Scheme 2*)**. The conventional process of synthesis of 8-Formyl compounds 4**(a-d)**, possess several steps, which needed skilled workup procedures. Therefore the MW synthesis of **4(a-d)** was done which forms the clean and green synthesis of the starting material. The 8-imino compounds synthesized were in moderate to good yields. The structures of synthesized Schiff' base analogs were confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectra. The ^{1}H-NMR spectra of Schiff bases **5(a-p)** showed the absence of a signal around δ 10 for the aldehyde proton, and the appearance of a signal at about δ 8.9 for HC=N. Furthermore, the ^{13}C-NMR appearance of a signal at about δ 161 confirmed the desired structures.

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 bacteria and fungi strains and the minimal inhibitory concentrations (MICs) using dimethylsulfoxide (DMSO) as a solvent (negative) control. MICs of the tested compounds along with the positive and negative control were determined by varying the dilution of the same from 12.5µg/mL to 200 µg/mL. The anti-bacterial activity was done by measuring the zone of inhibition using paper disc diffusion method called Kirby-Bauer's method.[14] The method was adopted with some modifications for the prepared compounds. Each experiment was done in the triplet.

The antibacterial activities results **(*Table2*)** were compared with the standard reference *Streptomycin*. The negative control was found to be negligible (<0.002) for both type of strains. Among the synthesized compounds, **5b**, **5f** and **5g** showed good antibacterial activity against both bacterial strains. The compounds **5a, 5d** and **5j** showed good activity against *S.aureus*, whereas **5a**, **5c** and **5h** possess good activity against *E.Coli*.

Similarly, for antifungal activities, the zone of inhibition was measured in *mm*. The results **(*Table3*)** were compared with the standard reference *Clotrimazole.* The compound **5b**, **5f, 5g** showed comparatively good activity against both antifungal strains. Whereas **5a, 5c, 5d** and **5j** showed moderately good antifungal activities against *C.albicans* and

Compounds **5c, 5j** and **5h** possess moderate antifungal activities against *A.niger*.

From the results of the biological activities, **5f, 5j** containing chlorine and **5g, 5c** having bromine and in the 6th position possess good antibacterial activities. Further in the compound **5f** and **5g** the presence of nitro (electron withdrawing) group may enhance the antagonist property. Compound **5k**, **5l, 5d** containing methyl group showed moderate potency against antifungal *C. albicans* and *A. niger*. Further observations of the activities of the amines with an aromatic ring and without aromatic rings infer that the former possess more activity than the latter. This may be due to the steric stability. 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.

4. CONCLUSION

The Novel compounds were designed and were tracked for diffusion into the biological system by the study of their CLogP, CMR, Mol. weight and tPSA values. After synthesis, these compounds were evaluated for *invitro* antimicrobial activities. These novel compounds showed good correlations with the assumed activities as per physicochemical data. And these compounds may show good activities even in in-vivo studies too. Many times the drugs were failed to show *in Vivo* activities due to low or slow diffusion into the biological system and thereby rejected in the course of their clinical trial 1 or 2. So, it is worth to test the compounds for their diffusivity well in advance with the available methods.

5. ACKNOWLEDGMENTS:

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Table 1
Some Physicochemical properties of the novel compounds.

Compd	CLogP	CMR	Mol.Wt.	tPSA(A^{o2})
5a	4.56112	9.9936	341.36	58.89
5b	4.98048	10.485	375.80	58.89
5c	5.18048	10.7706	420.26	58.89
5d	5.01012	10.4574	355.29	58.89
5e	2.80112	10.4911	386.36	102.59
5f	3.22048	10.9825	420.80	102.59
5g	3.42048	11.2681	465.25	102.59
5h	3.25012	10.9549	400.38	102.59
5i	5.06012	10.4574	355.39	58.89
5j	5.47948	10.9488	389.83	58.89
5k	5.67948	11.2344	434.28	58.89
5l	5.50912	10.9212	369.41	58.89
5m	2.1835	8.1551	280.28	84.91
5n	2.67038	8.6465	314.72	84.91
5o	2.87038	8.9321	359.17	84.91
5p	2.6325	8.6189	294.3	84.91

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	0.05	0.025	0.0125	0.2mL	0.1	0.05	0.025	0.0125
5a	22	21	19	17	15	26	25	24	22	20
5b	24	22	20	18	16	23	22	21	21	20
5c	20	19	17	15	10	23	22	21	20	20
5d	22	20	18	16	15	20	20	18	17	17
5e	19	16	15	14	12	16	14	9	8	6
5f	23	22	20	18	17	27	25	23	22	19
5g	24	22	18	17	15	23	21	21	19	18
5h	23	22	20	18	17	24	22	21	18	16
5j	20	19	18	16	14	17	16	15	8	-
5k	19	18	17	15	13	19	17	16	16	12
5l	18	16	15	-	9	19	18	17	16	15
Streptomycin	**22**	**20**	**18**	**17**	**16**	**25**	**22**	**22**	**20**	**20**

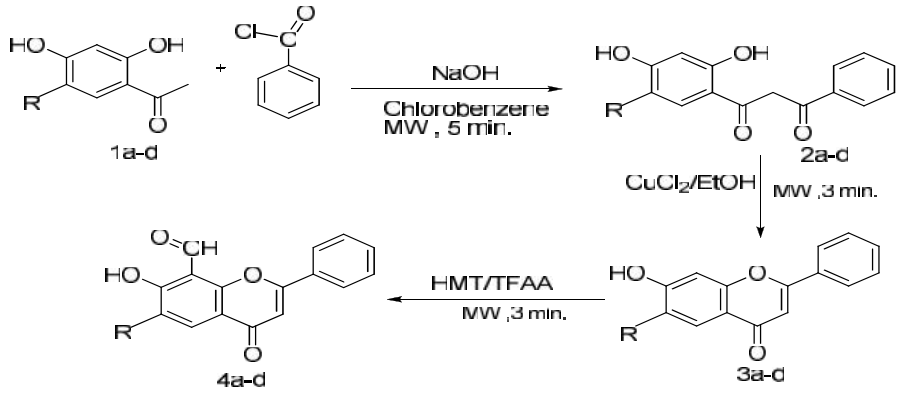
*Concentration: 0.2mL = 200µg/mL , - : zone of inhibition < 6mm, DMSO-<0.02*mm*

Table3
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	0.2mL	0.1	0.05	0.025	0.0125
5a	19	17	15	14	12	17	14	13	9	-
5b	23	21	19	17	15	23	22	20	18	15
5c	19	17	15	12	10	23	21	20	19	17
5d	19	14	14	11	10	16	15	14	12	10
5f	23	20	18	16	14	23	21	19	17	13
5g	23	21	20	18	16	25	22	20	18	16
5h	23	21	20	17	16	22	20	18	17	16
5j	22	18	16	12	11	19	18	16	14	-
5l	17	15	12	11	10	18	16	15	14	10
5k	17	15	12	11	-	18	17	18	16	12
Clotrimazole	**18**	**16**	**15**	**13**	**12**	**20**	**19**	**17**	**16**	**12**

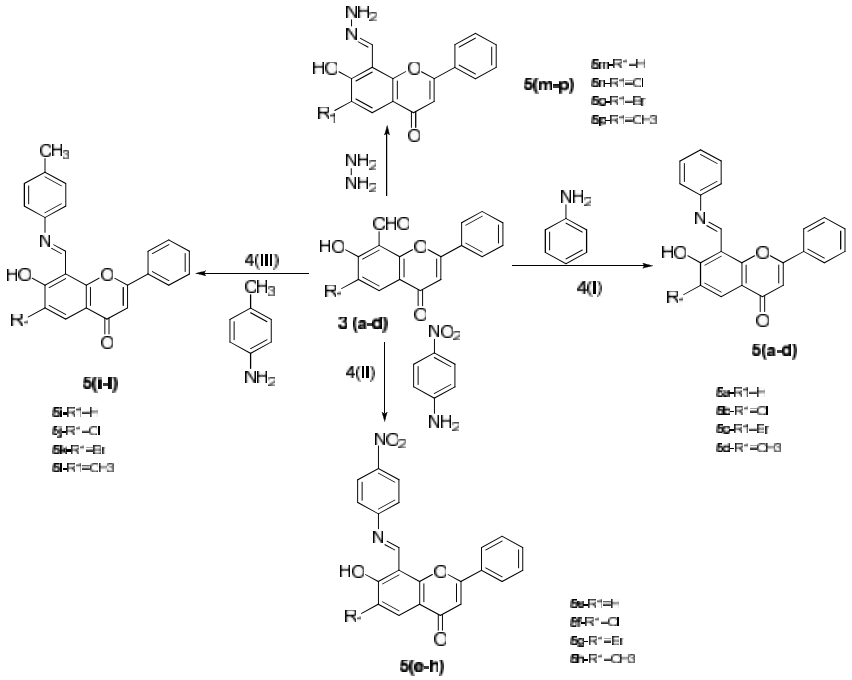
*Concentration: 0.2mL = 200µg/mL , - : zone of inhibition < 6*mm*, DMSO-<0.02*mm*

Scheme 1:

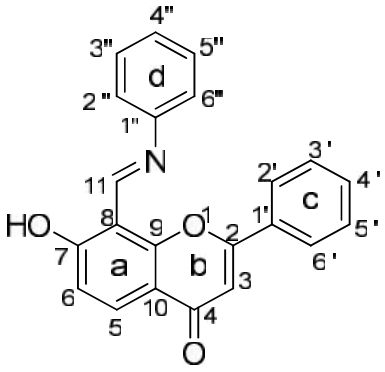


Scheme1 showing the preparation of 8-formyl-7-hydroxy-2-phenyl chromones

Scheme 2:



Scheme 2: showing the synthesis of novel Schiff's base of 8-formyl-7-hydroxy-2-phenyl chromones 5(a-p)



5a

Fig. 1
General structure of the Schiff's base of 8-formyl-7-hydroxy-2-phenyl chromones

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