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Design, Synthesis and Pharmacological Evaluation of Chromenones and Related Analogues

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Abstract: Two chalcones (**4a, 4b**) and nine schiff bases (**4c – 4k**) of 7-hydroxy-3-formyl chromen-4-one have been synthesized and characterized on the basis of IR, NMR and elemental analysis. All the synthesized compounds were evaluated for antimicrobial activity against both gram positive and gram negative organisms. The Schiff base synthesized from 2,4-dinitro phenyl hydrazine (**4h**) have shown the significant antimicrobial activity.
Keywords: Chromenone, Chalcones, Schiff base, Antimicrobial.

Introduction

Oxygen containing heterocycles are abundantly found in nature[1]. Flavone, isoflavones, flavanones, catechins, anthocyanins are some phytoconstituents collectively grouped as flavonoids and isoflavonoids. Chemically they are categorized as chromenes, chromenones, dihydrofurobenzofurans, chromanochromanones, benzofurochromans, xanthones and amphipyrones. Chromenones are naturally occurring compounds possessing diverse biological and pharmacological activities. Many synthetic analogues of chromenones have been evaluated for their anticancer[2-4], anticonvulsant[5], angioprotective, antiallergic, antihistaminic[6], antimicrobial[7], antioxidant[8], anti-HIV[9]. Due to emergence of multi-drug-resistant strains of microbes[10] like methicillin resistant staphylococcus aureous (MRSA), vancomycin resistant enterococci (VRE), multidrug resistant mycobacterium tuberculosis (MRD-TB) and penicillinase producing neisseria gonorrhoeae (PPNG), microbial diseases have become more complex to tackle. Many synthetic and semi-synthetic antimicrobial drugs have been discovered and used in clinical practice. In spite of significant developments in antimicrobial therapy, the problem of drug resistance, spectrum of activity, potency, safety and toxicity remain unresolved. Many quinolones and fluoroquinolones like Norfloxacin, Lomefloxacin, Enoxacin, Ofloxacin, Ciprofloxacin, Levofloxacin, Sparfloxacin, Gatifloxacin, Moxifloxacin, Garenoxacin and Moxifloxacin are used in clinical practice. Structurally 4-quinolones and 4H-chromen-4-ones are very similar in many respects. Calchones[11] and schiff's bases[12] of heterocyclic compounds are also versatile molecules possessing antimicrobial activity. Based on above impetus we attempted the synthesis of chalcones and schiff bases of 4H-chromen-4-ones (benzopyran-4-ones). 7-Hydroxy-3-formyl chromen-4-one was synthesized from 2,4-dihyroxy acetophenone by reported method[13]. Two calchones with various aromatic ketones (**4a, 4b**) were synthesized by claisen-schmidt condensation of 7-hydroxy-3-formyl chromen-4-one with substituted acetophenones by base catalyzed reaction followed by dehydration and nine schiff bases (**4c-4k**) were prepared. All the synthesized chalcones and Schiff bases were evaluated for their antimicrobial activity against two Gm+ organisms (*Staphylococcus aureus, Bacillus subtilis*) and two Gm-ve organisms (*Escherichia coli, Pseudomonas aeruginosa*).

Experimental

Melting points of the synthesized compounds was determined by using Veego melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellet method in the range of 4000-500 cm^{-1} on Shimadzu IR-Affinity 1800 Fourier Transform IR Spectrophotometer, and frequencies were recorded in wave numbers. ^1H NMR (400 MHz) spectra was recorded on Varian Mercury-300 NMR spectrometer using $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) down field from internal reference TMS. Purity of the compounds were checked by thin layer chromatography using silica gel-G coated aluminium plates (Merck, 60F-254) as stationary phase, n-hexane : ethyl acetate as mobile phase.

Preparation of 2,4-dihydroxyacetophenone (Resacetophenone)[14]

2,4-dihydroxyacetophenone was synthesized by reported method. Briefly, the synthesis was carried out by dissolving freshly fused and powdered zinc chloride (0.24 mole) in 32 ml of glacial acetic acid by heating in sand bath. Dry resorcinol (0.2 mole) was added with stirring at 140 ^0C. The solution was heated until it just begins to boil and kept for 20 minutes at 150 ^0C. Dilute HCl (1:1) was added to the mixture and the solution was cooled to 5 ^0C. The separated product was filtered and washed with dilute HCl. The product was recrystallised from hot water. The physical data of synthesized compounds are **given in Table 1.**

Preparation of 7-hydroxy-3-formyl chromen-4-one

In dry DMF (60 ml) in three neck flask, $POCl_3$ (37.5 ml) was added slowly with vigorous stirring at 50 ^0C. Heating and stirring was continued for 2 hrs at 45-55 ^0C. The solution of resacetophenone (9.12 gm) in DMF (12.5 ml) was then slowly added with stirring at 50 ^0C and stirring was continued for 2 hrs. After cooling the mixture was kept overnight at room temperature and diluted slowly by adding ice cold water (250 ml) and was stirred againg for 6hrs. The red crystalline product separated was filtered and recrystallised from alcohol.

Preparation of chalcones (4a, 4b)

Chalcones were prepared by reaction of equimoles of 7-Hydroxy-3-formyl chromen-4-one and substituted acetophenones. 7-Hydroxy-3-formyl chromen-4-one (0.01 mole) was dissolved in 5 ml methanol. Substituted acetophenone (0.01 mole) was added with constant stirring. 10 ml of 50% NaOH was added to it with stirring over 10-15 minutes. The mixture was poured over crushed ice with stirring. The resulting solution was acidified with concentrated HCl. The product obtained was filtered and recrystallised from methanol. Spectral data of synthesized compounds is **given in table 2.**

Preparation of Schiff bases (4c – 4k)

Schiff bases were prepared by reaction of equimoles of 7-Hydroxy-3-formyl chromen-4-one and various amines. 7-Hydroxy-3-formyl chromen-4-one (0.01 mole) was dissolved in 5 ml methanol. Amine (0.01 mole) was added with constant stirring. To the resulting mixture 2-4 drops of concentrated H_2SO_4 was added and the mixture was refluxed for 1-2 hrs. After completion of reaction mixture was poured over crushed ice with stirring. The product obtained was filtered and recrystallised from methanol.

Spectral characteristics of synthesized compounds

4a: FT-IR (KBr pellet, cm^{-1}) 3221 (O-H str), 1600 (C=C str), 1640 (C=O str), ^1H NMR ($CDCl_3$, δ ppm) 6.279 (d, -CH=, 1H), 6.61 (d, Ar-H, 1H), 6.85 (d, Ar-H, 1H), 7.04-7.96 (m, Ar-H, 6H), 7.72 (d, -CH=, 1H), 7.76 (d, -CH=, 1H), 5.35 (s, -OH, 1H); Anal Calcd. for $C_{18}H_{12}O_4$: C (73.97%), H (4.14%), O (21.9%); Found: C (73.87%); H (4.15%); O (21.68%).

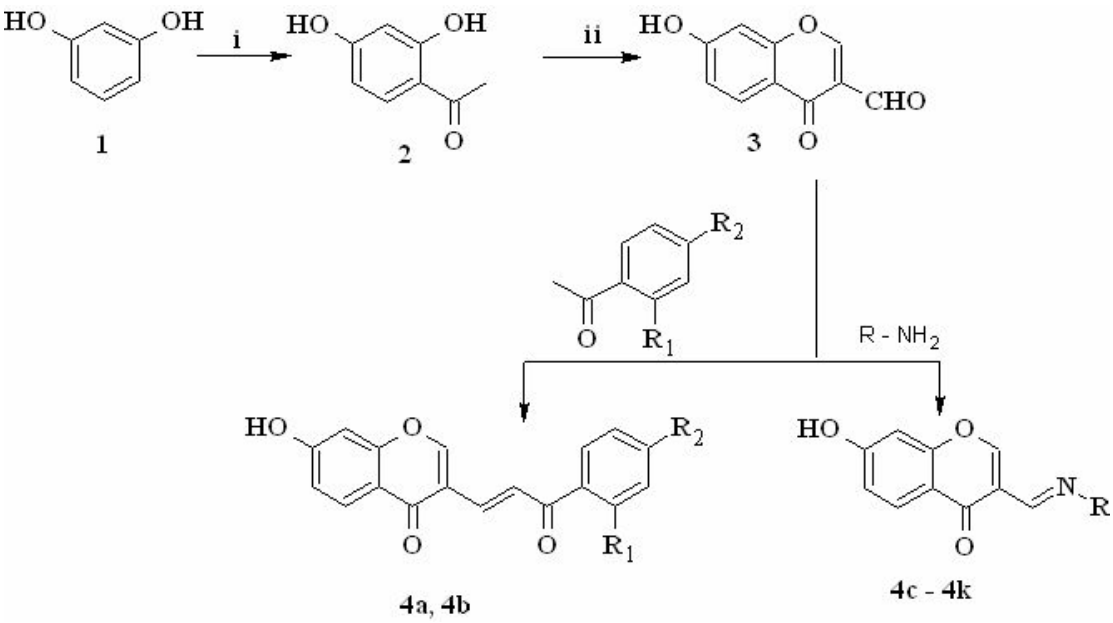
4b: FT-IR (KBr pellet, cm^{-1}) 3302 (O-H str), 1606 (C=C str), 1701 (C=O str), ^1H NMR ($CDCl_3$, δ ppm) 5.35 (s, -OH, 3H), 6.38-6.69 (m, Ar-H, 5H), 7.22 (d, -CH=, 1H), 7.76 (d, -CH=, 1H0, 7.96 (m, Ar-H, 2H); Anal Calcd. for $C_{18}H_{12}O_6$: C (66.67%), H (3.73%), O (29.6%); Found: C (65.71%); H (4.15%); N (30.14%).

4c: FT-IR (KBr pellet, cm^{-1}) 3446 (O-H str), 1620 (C=O str), 1510 (C=N str) 3309 (N-H str) ^1H NMR ($CDCl_3$, δ ppm) 5.4 (s, -OH, 1H), 6.45-7.96 (m, Ar-H, 4H), 7.0 (s, -NH-, 1H), 8.56 (s, -NH2, 2H), 8.83 (s, -CH=, 1H); Anal Calcd. for $C_{11}H_9N_3O_3S$: C (50.18%), H (3.45%), N (15.96%), O (18.23%), S (12.18%) ; Found: C (51.56%); H (4.15%); N (15.80%); O (18.79%); S (9.7%).

4d: FT-IR (KBr pellet, cm^{-1}) 3385 (O-H str), 1625 (C=O str), 1625 (C=N str) 3221 (N-H str) ^1H NMR ($CDCl_3$, δ ppm) 5.3 (s, -OH, 1H), 6.45-7.96 (m, Ar-H, 4H), 7.0 (s, -NH-, 1H), 6.0 (s, -NH2, 2H), 8.83 (s, -CH=, 1H); Anal Calcd. for $C_{11}H_9N_3O_4$: C (53.44%), H (3.67%), N (17%), O (25.89%); Found: C (53.89%); H (4.10%); N (16.99%); O (25.02%).

4e: FT-IR (KBr pellet, cm^{-1}) 3219 (O-H str), 1618 (C=O str), 1610 (C=N str), ^1H NMR ($CDCl_3$, δ ppm) 5.3 (s, -OH, 1H), 6.45-7.96 (m, Ar-H, 4H), 7.0 (s, -NH-, 1H), 6.0 (s, -NH2, 2H), 8.83 (s, -CH=, 1H); Anal Calcd. for $C_{10}H_8N_2O_3$: C (58.82%), H (3.95%), N (13.72%), O (23%); Found: C (57.78%); H (4.15%); N (14.10%); O (23.97%).

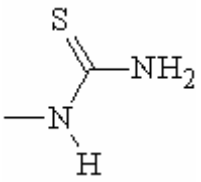
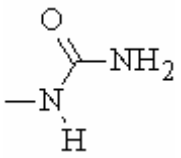
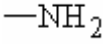
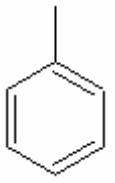
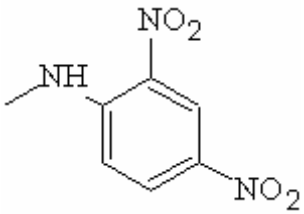
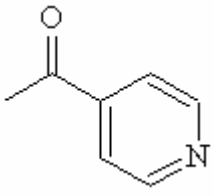
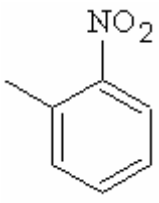
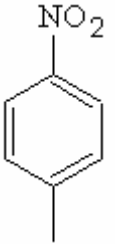
Scheme of synthesis



i: $ZnCl_2$/Glacial acetic acid, ii: DMF/$POCl_3$

1: Resorcinol **2**: 2,4-dihydroxyacetophenone **3**: 7-Hydroxy-3-formyl chromen-4-one

Chalcones: Schiff bases: R=

	R_1	R_2				
4a	-H	-H	**4c**		**4d**	
4b	-OH	-OH				
4e			**4f**			
4g			**4h**			
4i			**4j**			
4k						

4f: FT-IR (KBr pellet, cm^{-1}) 3400 (O-H str), 1624 (C=O str), 1508 (C=N str), ^1H NMR ($CDCl_3$, δ ppm) 5.35 (s, -OH, 1H), 6.45-6.69 (m, Ar-H, 2H), 7.0 (s, -NH2, 2H), 7.58-7.96 (m, Ar-H, 2H), 6.8 (s, -CH=, 1H), 9.1 (s, -OH, 1H); Anal Calcd. for $C_{10}H_7NO_4$: C (58.54%), H (3.44%), N (6.83%), O (31.19%); Found: C (58.50%); H (4.15%); N(6.83%); O (30.52%)

4g: FT-IR (KBr pellet, cm^{-1}) 3446 (O-H str), 1624 (C=O str), 161508 (C=N str), ^1H NMR ($CDCl_3$, δ ppm) 5.4 (s, -OH, 1H), 6.45-7.96 (m, Ar-H, 9H), 7.5 (s, -CH=, 1H); Anal Calcd. for $C_{16}H_{11}NO_3$: C (72.45%), H (4.18%), N (5.28%), O (18.09%); Found: C (71.56%); H (4.15%); N (4.10%); O (20.19%).

4h: FT-IR (KBr pellet, cm^{-1}) 3400 (O-H str), 1650 (C=O str), ^1H NMR ($CDCl_3$, δ ppm) 5.35 (s, -OH, 1H), 6.45-7.9 (m, Ar-H, 5H), 7.0 (s, -NH-, 1H), 8.83 (s, -CH=, 1H), 8.4 (s Ar-H, 1H), 8.8 (d, Ar-H, 1H); Anal Calcd. for $C_{16}H_{10}N_4O_7$: C (51.9%), H (2.72%), N (15.13%), O (30.25%); Found: C (50.65%); H (3.11%), N (15.56%), O (30.68%).

4i: FT-IR (KBr pellet, cm^{-1}) 3275 (O-H str), 1699 (C=O str), 1471 (C=N str), ^1H NMR ($CDCl_3$, δ ppm) 5.35 (s, -OH, 1H), 6.45-7.9 (m, Ar-H, 6H), 7.5 (s, -CH=, 1H), 8.89 (d, Ar-H, 2H); Anal Calcd. for $C_{16}H_{10}N_2O_4$: C (65.31%), H (3.43%), N (9.52%), O (21.75%); Found: C (65.98%); H (4.15%) ; N (9.88%); O (19.99%).

4j: FT-IR (KBr pellet, cm^{-1}) 3387 (O-H str), 1625 (C=O str), 1504 (C=N str), ^1H NMR ($CDCl_3$, δ ppm) 5.35 (s, -OH, 1H), 6.45-7.9 (m, Ar-H, 6H), 7.5 (s, -CH=, 1H), 8.0 (d, Ar-H, 1H); Anal Calcd. for $C_{16}H_{10}N_2O_5$: C (61.94%), H (3.25%), N (9.03%), O (25.78%); Found: C (61.99%); H (3.30%); N (9.03%); O (25.68%).

4k: FT-IR (KBr pellet, cm^{-1}) 3483 (O-H str), 1631 (C=O str), 1504 (C=N str), 1597 (N-O str), ^1H NMR ($CDCl_3$, δ ppm) 5.35 (s, -OH, 1H), 6.45-7.9 (m, Ar-H, 6H), 7.5 (s, -CH=, 1H), 8.0 (d, Ar-H, 2H); Anal Calcd. for $C_{16}H_{10}N_2O_5$: C (61.94%), H (3.25%), N (9.03%), O (25.78%); Found: C (61.93%); H (3.20%); N (9.12%); O (25.75%).

Antimicrobial activity

The antimicrobial activity of all the synthesized compounds (**4a – 4k**) were examined against different Gram-positive (*Bacillus subtilis and Staphylococcus aureus*) and Gram-negative (*Escherichia coli and Pseudomonas aeruginosa*) by measuring zone of inhibition. The antimicrobial activity was performed by agar cup plate method at the concentration level of 100µg/ml. Streptomycin was used as standard drug at a concentration of 100µg/ml. Nutrient agar was used as culture media for antibacterial activity. 24 hrs old culture of bacterial pathogen was placed in nutrient agar and spread throughout the plate by spread plate technique. Wells were bored using sterile borer at equidistance. The plates were kept at room temperature for 30 minutes. The test compounds, standard and control was placed in respective wells and plates were incubated at 37 ^{0}C for 36 hrs. Zone of inhibition was measured by zone reader. The results are given in table 2.

Evaluation of physical properties

Computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, TPSA and other simple molecular descriptors. Each structure was fully geometry optimized using the Chem 3D Pro 11.0 by MM2 force field. Various molecular descriptors were then computed by using molinspiration[15] tool and TSAR software. The data is given in Table 1.

Result and Discussion

All the synthesized compounds have been characterized by IR, ^1H NMR spectral data and elemental analysis and were evaluated for their antimicrobial activity against Gram positive and Gram negative organisms. Compound **4h** showed significant antimicrobial activity against all test organisms. From the physical properties computed it was found that compound **4h** has more total polar surface area and higher log P value. Thus TPSA and log P values may be contributing towards the better antimicrobial activity. Compounds **4b, 4g, 4j** and **4k** showed moderate antimicrobial activity against test organisms. Compounds **4d, 4e** and **4i** showed poor activity against test organisms. Chalcone **4a** was found more effective than **4b** and lipophilicity may be the contributing factor in this case. This research work reveals that the chalcones and schiff bases of chromenone possess antimicrobial activity.

Acknowledgement

I would like to express my gratitude to Prof. M. N. Navale, Founder President, Sinhgad Technical Education Society, Pune and Principal, Smt. Kashibai Navale College of Pharmacy, for providing the necessary facilities during the course of this research work.

Table 1: Physical Data of Synthesized compounds

Comp	M.F.	M.W.	M.P./(^{0}C)	%yield	R_f	LogP	TPSA	HBA	HBD	NRB	MR	MSA	TD
4a	$C_{18}H_{12}O_4$	292.3	260 - 262	41.3	0.60	2.93	67.51	4	1	3	83.11	276.99	3.69019
4b	$C_{18}H_{12}O_6$	324.28	140 - 143	81.4	0.82	2.37	107.96	6	3	3	86.50	290.416	3.34786
4c	$C_{11}H_9N_3O_3S$	263.27	265 -267	52.0	0.49	1.18	100.85	6	4	3	62.59	245.212	2.62773
4d	$C_{11}H_9N_3O_4$	247.20	250 - 260	52.1	0.51	0.64	117.92	7	4	2	62.48	232.073	1.04122
4e	$C_{10}H_8N_2O_3$	204.18	258 - 259	77.7	0.43	0.46	88.82	5	3	1	55.43	200.279	2.12523
4f	$C_{10}H_7NO_4$	205.16	270 - 272	72.2	0.57	1.66	83.03	5	2	1	52.42	196.3	3.56534
4g	$C_{16}H_{11}NO_3$	265.26	280 - 285	58.3	0.67	2.77	62.80	4	1	2	75.62	248.085	4.83744
4h	$C_{16}H_{10}N_4O_7$	370.27	200 - 203	50.9	0.37	3.91	166.47	11	2	5	95.35	320.402	5.19626
4i	$C_{16}H_{10}N_2O_4$	294.26	236 - 238	62.9	0.70	0.82	92.76	6	1	2	78.49	267.367	5.40003
4j	$C_{16}H_{10}N_2O_5$	310.26	55 - 57	71.6	0.46	2.68	108.62	7	1	3	82.94	265.497	6.3218
4k	$C_{16}H_{10}N_2O_5$	310.26	160 - 165	81.9	0.45	2.73	108.62	7	1	3	82.94	271.441	5.93207

M.F.: Molecular formula M.W.: Molecular weight M.P.: Melting point R_f: Retention factor in TLC Log P: Octanol-water partition coefficient
TPSA: Total polar surface area HBA: Hydrogen bond acceptor HBD: Hydrogen bond donor NRB: Number of rotatable bonds
MR: Molar refractivity MSA: Molecular surface area TD: Total dipole

Table 2: Antimicrobial activity data of synthesized compounds

Compound	Concentration (µl/ml)	Zone of inhibition in mm			
		B. subtilis	E. coli	S. aureus	P. aureginosa
4a	100	20	15	16	14
4b	100	17	12	14	13
4c	100	17	16	17	18
4d	100	14	13	17	14
4e	100	17	14	14	14
4f	100	17	16	17	15
4g	100	16	15	15	15
4h	100	23	21	19	18
4i	100	15	16	15	14
4j	100	19	22	17	19
4k	100	14	16	18	14
Streptomycin	100	26	27	25	24

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