Research Article

Design, Synthesis and Pharmacological Evaluation of Chromenones and Related Analogues

RB. Patil*, SD. Sawant and VB. Iyar

Sinhgad Technical Education Society's, Smt. Kashibai Navale College of Pharmacy, Kondhwa (Bk), Pune, Maharashtra, India.

ABSTRACT

Two chalcones (**4a, 4b**) and nine schiff bases (**4c – 4k**) of 4-methyl-2,6-dioxo-2H,6H-pyrano[3,2-g]chromene-8-carbaldehyde 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 (*Bacillus subtilis and Staphylococcus aureus*) and Gram-negative (*Escherichia coli and Pseudomonas aeruginosa*) organisms by measuring zone of inhibition. The Schiff base synthesized from 2,4-dinitro aniline (**4k**), *p*-nitroaniline (**4e**) and α-naphthylamine (**4f**) have shown the significant antimicrobial activity.

Keywords: Pyrano[3,2-g]chromene, 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, chromano chromanones, 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 chromenones fused with coumarin ring. During the ring construction of chromenone ring Heck[13] reaction gave 8-formyl group substituent which was further elaborated to get different chalcones and Schiff bases. The calchones with various aromatic ketones (**4a, 4b**) were synthesized by claisen-schmidt condensation of 4-methyl-2,6-dioxo-2H,6H-pyrano[3,2-g]chromene-8-carbaldehyde 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 7-hydroxy-4-methyl coumarin (1)[14]

7-hydroxy-4-methyl coumarin was synthesized by reported method. Briefly, the synthesis was carried out by adding conc. Sulphuric acid 80 ml in a ice cold (0-5^{0}C) mixture of resorcinol (0.2 mole) and ethylacetoacetate (0.2 mole). The mixture was kept at room temperature overnight and poured over crushed ice. The solid obtained was filtered and washed with water. The product was recrystallised from ethanol. The physical data of synthesized compounds are given in Table 1.

Preparation of 6-acetyl-7-hydroxy-4-methylcoumarin

Freshly fused and powdered zinc chloride (13.629 g, 0.1 mole) was dissolved in glacial acetic acid (12 ml) by heating in beaker on a sand bath. Dry compound 7-Hydroxy-4-methyl coumarin (14.608 g, 0.083 mole) was added with stirring to the mixture at 140^{0}C . The solution is heated until it just begins to boil and kept for 20 min. at 150^0 C. Dilute hydrochloric acid (1:1, 100 m) was added to the mixture. The separated product was filtered and washed with dilute hydrochloric acid (1:3). It was recrystallized from hot water containing little hydrochloric acid . Yield 10.9 g (93.9%) M.P. 211^{0}C

Preparation of 4-methyl-2,6-dioxo-2H,6H-pyrano[3,2-g]chromene-8-carbaldehyde

In dry DMF (12.5 ml) in three neck flask $POCl_3$ (7.5 ml, 0.049 mole) was added slowly with vigorous stirring at 50^{0}C. Heating and stirring was continued for 2 hrs at 45-55^{0}C. The solution of 6-acetyl-7-hydroxy-4-methyl-coumarin (2.616 g, 0.012 mole) in DMF (2.5 ml) was then slowly added with stirring at 50^{0}C 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 again for 6 hrs. The brownish crystalline product separated was filtered and recrystallised from alcohol. Yield 3.7 g (82.2%) M.P. 158 - 160^{0}C

Preparation of chalcones (4a, 4b)

Chalcones were prepared by reaction of equimoles of 4-methyl-2,6-dioxo-2H,6H-pyrano[3,2-g]chromene-8-carbaldehyde and substituted acetophenones. 4-methyl-2,6-dioxo-2H,6H-pyrano[3,2-g]chromene-8-carbaldehyde (2.56 g, 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 4-methyl-2,6-dioxo-2H,6H-pyrano[3,2-g]chromene-8-carbaldehyde and various amines. 4-methyl-2,6-dioxo-2H,6H-pyrano[3,2-g]chromene-8-

carbaldehyde (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}) 3076 (C-H str), 1068 (C-O str), 1600 (C=C str), 1640 (C=O str); ^1H NMR ($CDCl_3$, δ ppm) 2.433 (s, $-CH_3$, 3H), 6.7 (s, -CH=, 1H), 6.9 (s, -CH=, 1H), 7.1-7.9 (m, Ar=H, 7H), 5.9 (d, -CH=, 2H); Anal Calcd. for $C_{18}H_{12}O_4$: C (73.74%), H (3.94%), O (22.32%); Found: C (73.87%); H (4.00%); O (22.13%).

4b: FT-IR (KBr pellet, cm^{-1}) 3302 (O-H str), 1068 (C-O str), 1600 (C=C str), 1701 (C=O str); ^1H NMR ($CDCl_3$, δ ppm) 2.433 (s, $-CH_3$, 3H), 6.7 (s, -CH=, 1H), 6.9 (s, -CH=, 1H), 7.1-7.9 (m, Ar=H, 5H), 5.9 (s, -OH, 1H);, 5.908 (s, -OH, 1H); Anal Calcd. for $C_{18}H_{12}O_6$: C (67.69%), H (3.62%), O (28.69%); Found: C (67.71%); H (3.67%); O (28.62%).

4c: FT-IR (KBr pellet, cm^{-1}) 1608 (C=N str), 1695 (C=O str), 3076 (C-H str), 1068 (C-O str), 1386 (C-H def $-CH_3$); ^1H NMR ($CDCl_3$, δ ppm) 2.1 (s, $-CH_3$, 3H), 2.4 (s, -Ar-CH_3, 3H), 8.387 (s, -CH=, 1H), 6.7-7.2 (m, Ar-H, 6H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); Anal Calcd. for $C_{11}H_9N_3O_3S$: C (72.5%), H (3.95%), N (4.23%), O (19.32%) ; Found: C (72.47%); H (4.15%); N (4.80%); O (18.58%).

4d: FT-IR (KBr pellet, cm^{-1}) 3350 (O-H str), 1681 (C=O str), 1600 (C=N str) 3161 (C-H str) ^1H NMR ($CDCl_3$, δ ppm) 2.1 (s, $-CH_3$, 3H), 8.3 (s, -CH=, 1H), 6.7-7.2 (m, Ar-H, 6H), 8.2 (s, OH, 1H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); Anal Calcd. for $C_{11}H_9N_3O_4$: C (67.2%), H (3.49%), N (3.73%), O (25.58%); Found: C (67.39%); H (4.10%); N (3.67%); O (24.84%).

4e: FT-IR (KBr pellet, cm^{-1}) 1700 (C=O str), 1600 (C=N str), 2922 (C-H str); ^1H NMR ($CDCl_3$, δ ppm) 2.1 (s, $-CH_3$, 3H), 8.3 (s, -CH=, 1H), 6.7-7.2 (m, Ar-H, 6H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); Anal Calcd. for $C_{10}H_8N_2O_3$: C (63.83%), H (3.21%), N (7.44%), O (25.51%); Found: C (63.78%); H (3.47%); N (7.80%); O (24.95%).

4f: FT-IR (KBr pellet, cm^{-1}) 1690 (C=O str), 1600 (C=N str), 1319 (C-N str), 3076 (C-H str) ^1H NMR ($CDCl_3$, δ ppm) 2.1 (s, $-CH_3$, 3H), 8.3 (s, -CH=, 1H), 6.7-7.2 (m, Ar-H, 9H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); Anal Calcd. for $C_{10}H_7NO_4$: C (75.58%), H (3.96%), N (3.67%), O (16.78%); Found: C (75.60%); H (4.15%); N(3.78%); O (16.47%)

4g: FT-IR (KBr pellet, cm^{-1}) 1600 (C=N str), 1700 (C=O str), 3018 (C-H str) ^1H NMR ($CDCl_3$, δ ppm) 2.4 (s, $-CH_3$,1H), 6.7-7.3 (m, Ar-H, 9H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); Anal Calcd. for $C_{16}H_{11}NO_3$: C (72.5%), H (3.95%), N (4.23%), O (19.32%); Found: C (71.56%); H (4.15%); N (4.10%); O (20.19%).

4h: FT-IR (KBr pellet, cm^{-1}) 1620 (C=N str), 1683 (C=O str), 3012 (C-H str); ^1H NMR ($CDCl_3$, δ ppm) 2.4 (s, $-CH_3$,1H), 6.7-7.3 (m, Ar-H, 9H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H), 8.5 (s, $-NH_2$, 1H); Anal Calcd. for $C_{16}H_{10}N_4O_7$: C (69.36%), H (4.07%), N (8.09%), O (18.48%); Found: C (70.10%); H (4.22%), N (8.12%), O (17.56%).

4i: FT-IR (KBr pellet, cm^{-1}) 1620 (C=N str), 1683 (C=O str), 3012 (C-H str); ^1H NMR ($CDCl_3$, δ ppm) 2.4 (s, $-CH_3$,1H), 2.6 (s, $-OCH_3$, 3H), 6.7-7.3 (m, Ar-H, 9H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); Anal Calcd. for $C_{16}H_{10}N_2O_4$: C (69.8%), H (4.18%), N (3.88%), O (22.14%); Found: C (69.98%); H (4.15%) ; N (4.35%); O (21.52%).

4j: FT-IR (KBr pellet, cm^{-1}) 3387 (-NH str), 1625 (C=O str), 1504 (C=N str), ^1H NMR ($CDCl_3$, δ ppm) 2.4 (s, $-CH_3$,1H), 2.6 (s, $-OCH_3$, 3H), 7.3 (m, Ar-H, 2H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); 8.498 (s, $-NH_2$, 2H); Anal Calcd. for $C_{16}H_{10}N_2O_5$: C (65.88%), H (3.55%), N (5.49%), O (25.07%); Found: C (65.99%); H (3.30%); N (5.70%); O (25.01%).

4k: FT-IR (KBr pellet, cm^{-1}) 1620 (C=N str), 1683 (C=O str), 3012 (C-H str); ^1H NMR ($CDCl_3$, δ ppm) 2.4 (s, $-CH_3$,1H), 2.6 (s, $-OCH_3$, 3H), 6.7-7.3 (m, Ar-H, 9H), 5.9 (s, -CH=N, 1H), 6.8 (s, -CH=, 1H); Anal

Calcd. for $C_{16}H_{10}N_2O_5$: C (57.02%), H (2.63%), N (9.97%), O (30.38%); Found: C (58.01%); H (3.20%); N (9.12%); O (29.67%).

Antimicrobial activity

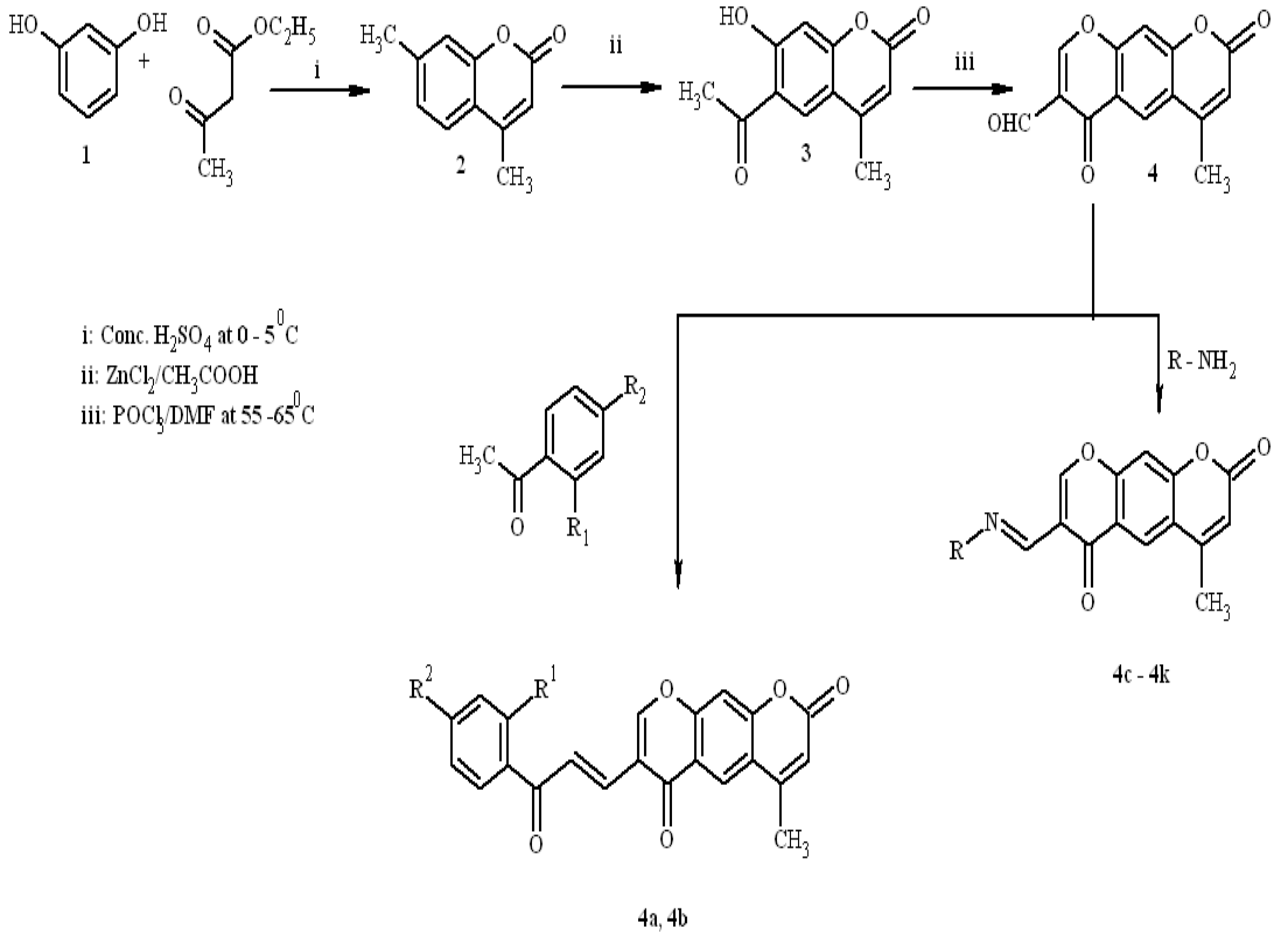
The antimicrobial activity of all the synthesized compounds (**4a – 4k**) were examined against different Gram-positive (*Bacillus subtilis and Staphylococcus aureus*) and Gram-negative (*Escherichia coli and Pseudomonas aeruginosa*) by measuring zone of inhibition. The antimicrobial activity was performed by agar cup plate method at the concentration level of 100µg/ml. Ciprofloxacin 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, PSA 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 online computational interface available at www.vcclab.org. The data is given in Table 1.

RESULT AND DISCUSSION

All the synthesized compounds have been characterized by IR, ^{1}H NMR spectral data and elemental analysis and were evaluated for their antimicrobial activity against Gram positive and Gram negative organisms. Compound **4k** showed significant antimicrobial activity against all test organisms. From the physical properties computed it was found that compound **4k** has more surface area, polar surface area, molar refractivity and highest hydrogen bond acceptor count. Thus molecular property of polarity and hydrogen acceptor sites in a molecule may be contributing towards the better antimicrobial activity. Compounds **4e** and **4f** showed moderate antimicrobial activity against test organisms. The rest of the compounds showed poor activity against test organisms. Chalcone **4b** was found more effective than **4a** 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 and further optimization of molecular property by can be undertaken.



Scheme of synthesis

Chalcones:

	R_1	R_2
4a	-H	-H
4b	-OH	-OH

Schiff bases:

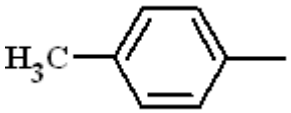
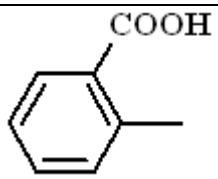
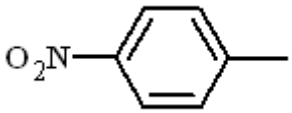
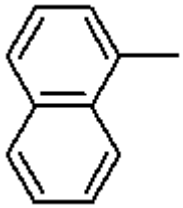
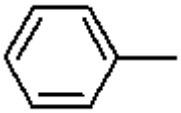
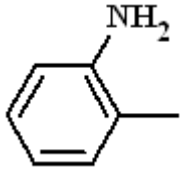
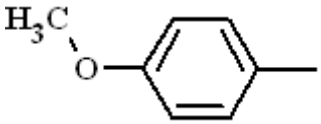
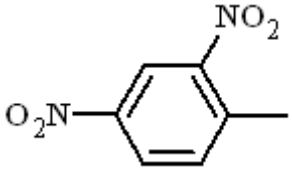
Compound	R=	Compound	R=
4c		4d	
4e		4f	
4g		4h	
4i		4j	-NH_2
4k			

Table 1: Physical Data of Synthesized compounds

Comp	M.F.	M.W.	M.P.(^{0}C)	%yield	R_f	LogP	MP	SA	PSA	HBD	HBA	RB	Ui	Hy	AMR	MlogP
4a	$C_{22}H_{14}O_5$	358.3	260 – 262	41.3	0.61	3.62	37.75	433	69.6	0	4	3	4.24	-0.806	101.3	3.136
4b	$C_{22}H_{14}O_7$	390.07	140 – 143	81.4	0.42	3.55	39.0	453.5	110.1	2	6	3	4.24	0.228	104.7	2.384
4c	$C_{20}H_{13}NO_4$	331.08	225-230	57.7	0.49	4.05	36.73	438.7	64.9	0	4	2	4.17	-0.798	98.05	3.256
4d	$C_{21}H_{13}NO_6$	375.07	240 – 242	72.9	0.31	3.31	37.33	442.9	102.2	1	6	3	4.28	-0.323	99.77	2.466
4e	$C_{20}H_{12}N_2O_6$	376.06	205 – 207	50.16	0.43	3.45	36.88	447.1	110.7	0	6	3	4.32	-0.707	100.3	2.852
4f	$C_{24}H_{15}NO_4$	381.10	260-262	47.12	0.56	4.51	42.48	469.1	64.9	0	4	2	4.52	-0.819	109.4	3.76
4g	$C_{20}H_{13}NO_4$	331.08	175-180	63.8	0.68	3.54	34.96	406.3	64.9	0	4	2	4.17	-0.79	93.01	3.033
4h	$C_{20}H_{14}N_2O_4$	346.09	195-198	57.7	0.47	2.61	36.11	420.6	90.9	1	5	2	4.17	0.251	97.71	2.261
4i	$C_{21}H_{15}NO_5$	361.09	236 - 238	33.4	0.70	3.40	37.49	454.2	74.1	0	5	3	4.17	-0.769	99.47	2.484
4j	$C_{14}H_9NO_4$	255.05	55 – 57	71.6	0.36	1.25	25.42	295.9	76.4	1	4	1	3.58	-0.249	68.34	1.541
4k	$C_{20}H_{11}N_3O_8$	421.05	160 - 162	68.9	0.47	3.35	38.87	487.6	156.6	0	8	4	4.45	-0.639	107.6	2.756

M.F.: Molecular formula M.W.: Molecular weight M.P.: Melting point R_f: Retention factor in TLC Log P: Octanol-water partition coefficient

MP: Molecular polarizability SA: Vander Waals surface area 3D PSA: Polar surface area HBD: Hydrogen bond donor count

HBA: Hydrogen bond acceptor count RB: Number of rotatable bonds Ui: Unsaturation index Hy: Hydroplilic factor AMR: Molar refractivity

MlogP: Moriguchi octanol-water partition coefficient

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	10	10	10	10
4b	100	12	15	10	10
4c	100	10	10	10	10
4d	100	12	10	10	10
4e	100	12	20	15	15
4f	100	10	20	12	12
4g	100	9	10	10	10
4h	100	15	15	10	10
4i	100	10	10	10	10
4j	100	9	10	10	10
4k	100	16	15	15	16
Ciprofloxacin	100	30	25	30	30
DMSO	0	0	0	0	0

CONCLUSION

The calchone and Schiff base analogues of chromen-4-ones (pyrano[3,2-g]chromene-8-carbaldehyde) synthesized were found promising antibacterial agents.

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