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PYRAZOLIDINE-3, 5-DIONE AND 2-METHYL-4-OXO-4H-THIOCHROMENE-8-CARBONYL CONJUGATES: SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING

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Keywords:

Pyrazolidine-3, 5-Dione, Thiochromen-4-one, Mercapto benzoic Acid, Antimicrobial, MIC

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ABSTRACT: In this study, a novel series of heterocyclic compounds containing pyrazolidine-3, 5-dione nucleus has been synthesized. The compounds were synthesized in four steps; esterification of 2-mercaptobenzoic acid in an acidic medium to yield 2-mercapto-benzoic acid ethyl ester which was cyclized using ethyl acetoacetate to form 2-methyl-4-oxo-4*H*-thiochromene-8-carboxylic acid ethyl ester. These were reacted phenyl hydrazine derivatives to give corresponding thiochromene derivatives, which were cyclized using diethylmalonate to obtain pyrazolidine-3, 5-dione derivatives. All the synthesized compounds were characterized by spectral (IR, NMR and MS) and elemental analysis. The compounds were screened for their antibacterial activity against Gram-positive bacteria (*B. subtilis*, S. *epidermidis, M. luteus*, S. *aureus, B. pumilis, and B. cereus*), Gram-negative bacteria (*K. pneumonia*, *E. coli*, and *P. aeroginosa*) and for antifungal activity against (*A. niger*, *C. albicans* and *F. solani*) by agar-diffusion method. The minimum inhibitory concentrations (MICs) of these compounds were also determined by tube dilution method. The antimicrobial effectiveness of all the compounds was found to be concentration dependent. Two compounds- 1-(3-chlorophenyl)-2-(2-methyl-4-oxo-4H-thiochromene-8- carbonyl) pyrazolidine-3, 5-dione **(5a)** and 1-(2-methyl-4-oxo-4H-thiochromene-8-carbonyl)-2-p-tolylpyrazolidine-3, 5-dione **(5d)** exhibited good antibacterial activity. The antibacterial activity of all the compounds was founds to better than the antifungal activity.

INTRODUCTION: Diseases caused by bacteria are among the leading causes of death worldwide. The obtain ability of limited number of antibiotics for the conduct of infections, and continuous improvement of resistance to the recently used antimicrobial agents, pose a grave challenge [1].

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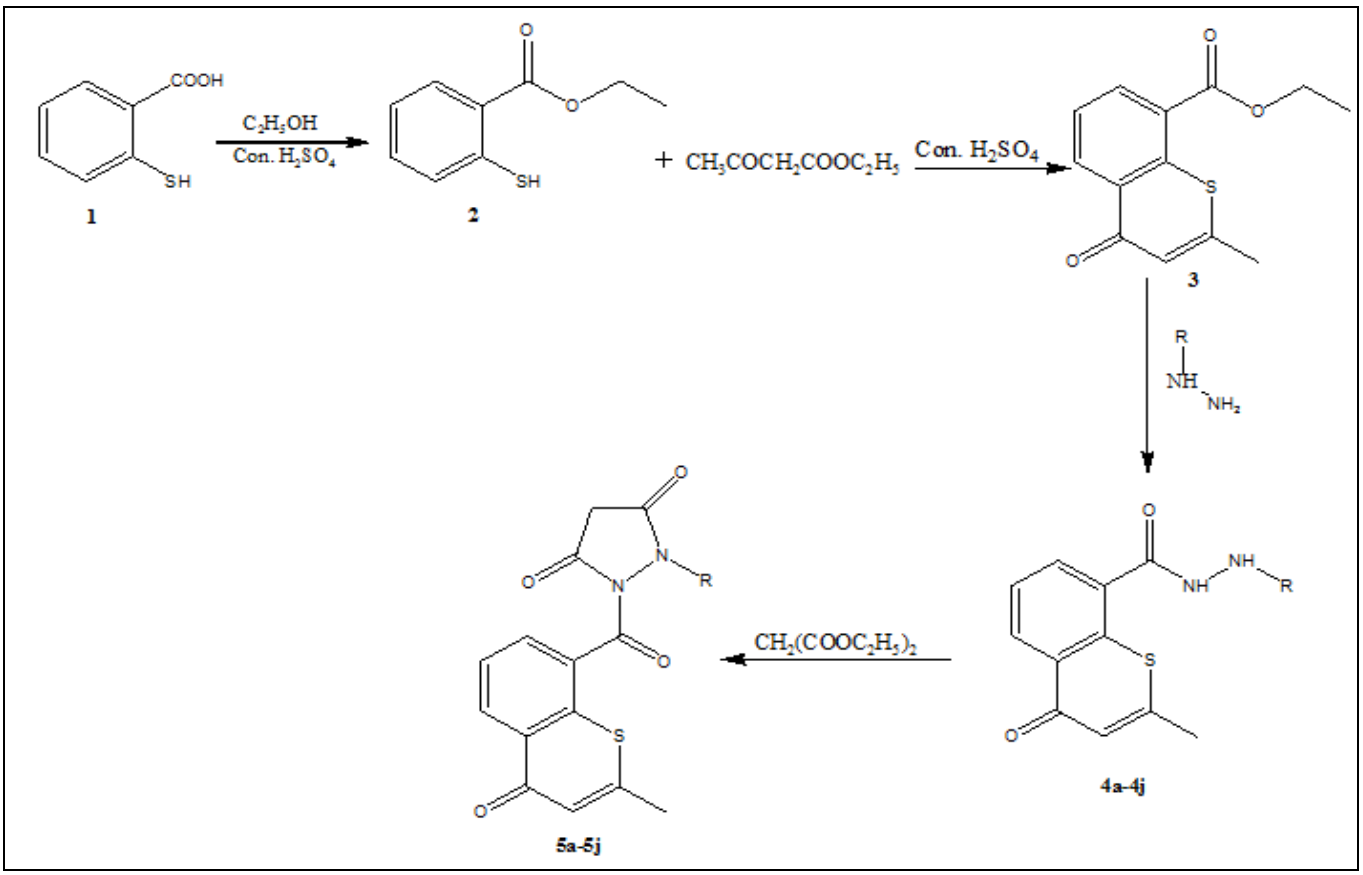
Thus, the sighting of innovative and intoxicating antimicrobial agents may be the only way to resolve the conflict problem and develop successful remedy for the treatment of infectious sicknesses. Pyrazolidine - 3, 5-dione contains; 5-member nitrogen heterocyclic scaffold like pyrazole have been received great attention by researcher in the recent years due to broad spectrum activities. [2] These pass diverse pharmacological and biological activities such as antimicrobial [3-14], anti-inflammatory [15-18], antidepressant [19-20] anti-cancer [21], anti-convulsant [22]. In the present study, the compounds are conjugates of two heterocyclic

moieties, *i.e.* Pyrazoline-3, 5-dione and thiochrome-4-one and are being investigated for their antimicrobial activity **(Scheme-1)**.



5a= R, 1-chloro-3-methyl-benzene
5b= R, 1,3-dichloro-5-methyl-benzene
5c= R, 1-chloro-3-fluoro-5-methyl-benzene
5d= R, p-xylene
5e= R, m-xylene
5f= R, 4-methyl-benzoic acid
5g= R, 1,2, 4-tribromo-5-methyl-benzene
5h= R, 1-methyl-3,5-dinitro-benzene
5i= R, 1,3,5-Triiodo-2-methyl-benzene
5j= R, 4-methyl-phenol

SCHEME 1: SYNTHESIS OF N-SUBSTITUTED PYRAZOLIDINE- 3,5-DINONE

MATERIALS AND METHODS:

Chemistry: All the reagents and chemicals used in the study were procured as LR grade from S.D. Fine Chem. Ltd., Mumbai and Sigma-Aldrich Chemical Ltd., India. Thin Layer Chromatography was used for monitoring the progress of the reactions and product formation. The thin layer chromatography of the synthesized compounds was carried out on Silica gel 60 F_{254}, E. Merck, Darmstadt, Germany with different solvent systems. Spots were detected under UV lamp (Short and Long Wavelength) and in an iodine chamber. The melting points were determined by open capillary method and are uncorrected. Infrared spectra (ν_{max} in cm^{-1}) of the synthesized compounds were recorded on Shimadzu FTIR-8400S in the range of 400-4000 cm^{-1} in potassium bromide. Mass spectra were recorded on VARIAN-500 instrument using Fast Atomic Bombardment (FAB) method at IIT Powai, India. ^{1}HNMR spectra (ppm, δ) and ^{13}C spectra (ppm, δ) were recorded on Bruker ADVANCE DRX 300 MHz/200 MHz spectrometer, with TMS as the internal standard. Microanalysis for C, H, and N were performed on an elementar Vario EL III at SAIF, Central Drug Research Institute, Lucknow, India. Turbidity measurements were made on a Shimadzu 1700 UV- Visible spectrophotometer.

General procedure for the synthesis of 2-Mercapto-benzoic acid ethyl ester (2): To a solution of 2-mercaptobenzoic acid (0.01 mol) (1) were dissolved in 80 ml of absolute ethanol and refluxed for about 2 hrs in the presence of few

drops of conc. Sulphuric acid. The reaction mixture was cooled and added 3.1 ml of con. sulphuric acid reflux for about 1 hr. The completion of reaction was checked by TLC using chloroform: methanol (95:5) as the solvent system. The reaction mixture was cooled and neutralized with sodium bicarbonate. The neutralized mixture was then poured into ice-water, filtered, dried and recrystallized using rectified spirit.

General procedure for the synthesis of 2-methyl-4-oxo-4H-thiochromene-8-carboxylic acid ethyl ester (3): To a solution of **2** (0.01 mol) were dissolved in 21.85 of ethyl acetoacetate and refluxed for about 3 hrs in the presence of 4-5 drops of con. sulphuric acid. The completion of reaction was checked by TLC using chloroform: methanol (98:2) as the solvent system. The reaction mixture was cooled and poured in ice-water, filtered, dried and recrystallized using rectified spirit.

General procedure for the synthesis of thiochrome derivatives (4a-4j): Equimolar quantities of 3 were dissolved in 10 ml of methanol; add appropriate phenyl hydrazine with 20 ml of ethanol. The reaction mixture was refluxed till the completion of reaction. The completion of reaction checked using different solvent system. Excess of ethanol was distilled off and poured in ice-water. The solid product was filtered, washed with ether, dried and recrystallized using rectified spirit.

General procedure for the synthesis of pyrazolidine-3, 5-dione derivatives (5a-5j): Equimolar quantities of thiochrome derivatives (4a-4j) and diethylmalonate were dissolved in 60 ml of ethanol in the presence of a few drops of acetic acid, and the reaction mixture was refluxed till the completion of reaction. The completion of the reaction (6-8 hrs) was checked by TLC using different solvent systems. The solid product was filtered, washed with water, dried and recrystallized using ethanol.

1-(3-chlorophenyl)-2-(2-methyl- 4 - oxo - 4H-thiochromene-8- carbonyl) pyrazolidine-3, 5-dione(5a): Light yellow crystal, yield 69.35%, melting range 124-126 °C; IR (KBr): 3040, 2950, 1735, 1705 and 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.9 (s, 3H), 4.42 (s, 2H), 6.96 (d, 2H), 7.2-7.4 (t, 1H), 7.5-7.62 (t, 3H,), 7.8-8 (t, 2H); ^{13}C NMR ($CDCl_3$, δ, ppm): 23.3 (1C, CH_3), 45.0 (1C, CH_2), 124.8, 126.9, 129.2, 131.4, 133.3, 135.6, 137.8, 139.9, 141.9, 143.8, 145.7, 147.6, 149.8, 154.8 (14C, Ar-C), 165.1, 168.3, 172.2, 178.8 (4C, C=O); ms: m/z 414[M+1]. *Anal.* Calcd. for $C_{20}H_{13}ClN_2O_4S$. C, 58.18; H, 3.17; N, 6.79. Found: C, 57.55; H, 3.20; N, 6.35.

1-(3, 5 - dichlorophenyl) – 2 -(2-methyl-4-oxo-4H-thiochromene-8- carbonyl) pyrazolidine - 3, 5-dione (5b): Colorless crystal, yield 78.15%, melting range 153-154 °C; IR (KBr): 3030, 2940, 1700 and 1270 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.22 (s, 3H), 4.8 (s, 2H), 7.23-7.45 (m, 5H), 7.9 (d, 1H), 8.2-8.3 (d, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm):178.7, 172.3, 168.0, 165.6 (4C, C=O), 149.0, 146.5, 144.2, 142.0, 139.8, 137.3, 135.1, 132.4, 130.2, 128.0, 125.9, 123.2 (14C, Ar-C), 43.0 (1C, -CO-CH2-CO), 23.2 (1C, CH_3). ms: m/z 447[M+1]. *Anal.* Calcd. for $C_{20}H_{12}Cl_2N_2O_4S$. C, 53.70; H, 2.70; N, 6.26. Found: C, 53.45; H, 2.55; N, 6.45.

1-(3-chloro-5-fluorophenyl)-2-(2-methyl - 4-oxo-4-H-thiochromene-8-carbonyl) pyrazolidine - 3, 5-dione (5c): Yellow crystal, yield 79.45%, melting range 188-189 °C; IR (KBr):3030, 2930, 1265, 1725 and 1700 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.1 (s, 3H), 4.62 (s, 2H), 6.9 (d, 1H), 7.0-7.1 (d, 1H), 7.2-7.4 (m, 4H), 7.5-7.7 (t, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 180.0, 173.0, 168.9, 166.2 (4C, C=O), 163.7, 151.1, 137.8, 136.0, 133.9, 131.8, 129.1, 126.5, 124.0, 121.3, 119.0, 116.8, 111.2, 108.3 (14C, Ar-C), 45.4(1C, -CO-CH_2-CO), 24.2 (1C, CH_3); ms: m/z 431[M+1]. *Anal.* Calcd. for $C_{20}H_{12}ClFN_2O_4S$. C, 55.76; H, 2.81; N, 6.50. Found: C, 55.15; H, 2.25; N, 6.25.

1-(2-methyl-4-oxo-4H-thiochromene-8-carbonyl) -2-p-tolylpyrazolidine-3, 5-dione (5d): Pale yellow crystal, yield 66.85%, melting range 138-140 °C; IR (KBr): 3040, 2960, 1710 and 1227 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.2 (s, 3H), 3.7 (s, 3H), 4.3 (s, 2H), 6.9 (d, 1H), 7-7.2 (m, 4H), 7.3-7.5 (d, 1H), 7.6-7.64 (t, 1H), 7.8-8.0 (d, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 177.8, 172.6, 167.5, 164.0 (4C, C=O), 153.4, 137.9, 135.5, 133.0, 131.1, 129.4, 127.2, 125.1, 122.3, 120.0, 117.9, (14C, Ar-C), 45.5(1C, -CO-CH2-CO), 24.0, 21.0 (2C, CH_3); ms: 394 [M+2], 393 [M+1], 392 (M+). *Anal.* Calcd. for

$C_{21}H_{16}N_2O_4S$. C, 64.27; H, 4.11; N, 7.14. Found: C, 64.45; H, 4.25; N, 7.35.

1-(2-methyl-4-oxo-4H-thiochromene-8-carbonyl) - 2 - m - tolylpyrazolidine-3, 5-dione (5e): Pale orange crystal, yield 74.45%, melting range 148-150 °C; IR (KBr): 3020, 2970, 1705 and 1236 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.25 (s, 3H, CH_3), 3.9 (s, 3H, CH_3), 4.6 (s, 2H, CH_2), 6.93 (d, 2H, Ar-H), 7-7.3 (m, 4H, Ar-H), 7.5-7.7 (t, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, δ, ppm): 179.5, 172.4, 169.0, 166.1 (4C, C=O), 151.9, 141.0, 138.8, 136.5, 134.4, 132.0, 129.9, 127.8, 125.5, 123.6, 121.8, 120.0, 117.9, 115.8 (14C, Ar-C), 46.2 (1C, -CO-CH_2-CO), 24.0, 21.3 (2C, CH_3); ms: m/z 394 (M+2), 393 (M+1). *Anal.* Calcd. for $C_{21}H_{16}N_2O_4S$. C, 64.27; H, 4.11; N, 7.14. Found: C, 64.55; H, 4.20; N, 7.25.

4-[2-(2-methyl - 4- oxo - 4H - thiochromene-8 carbonyl)-3, 5- dioxopyrazolidin-1-yl] benzoic acid (5f): Pale yellow crystal, yield 65.95%, melting range 240-242 °C; IR (KBr): 3438, 3080, 1764 and 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm):3.2 (s, 3H), 4.6 (s, 2H), 6.6-6.7 (d, 2H), 6.9 (d, 2H), 7.5-7.7 (t, 2H), 7.8-7.9 (d, 2H), 11.04 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 178.9, 173.2, 168.1, 166.1, 164.0 (5C, C=O), 152.2, 141.1, 139.0, 136.8, 134.7, 132.3, 130.1, 127.9, 126.0, 123.5, 121.2, 119.3 (14C, Ar-C), 45.7(1C, -CO-CH_2-CO), 24.2 (1C, CH_3); ms: m/z 423[M+1]. *Anal.* Calcd. for $C_{21}H_{14}N_2O_6S$. C, 59.71; H, 3.34; N, 6.63. Found: C, 59.57; H, 3.25; N, 6.20.

1-(2-methyl-4-oxo-4H-thiochromene-8-carbonyl) -2- (2, 4, 6- tribromo-phenyl) pyrazolidine-3,5-dione (5g): Light yellow crystal, yield 72.65%, melting range 271-273 °C; IR (KBr): 3068, 1685, 1623 and 1223 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.74 (s, 3H), 4.56 (s, 2H), 6.9-7.1 (d, 1H), 7.2- 7.4 (t, 1H), 7.6-7.64 (t, 1H), 7.8-8 (m, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 181.4, 176.0, 171.0, 168.9 (4C, C=O), 166.8, 154.0, 142.8, 140.6, 138.3, 136.4, 134.0, 132.9, 129.7, 127.6, 125.4, 123.0 (14C, Ar-C), 43.8(1C, -CO-CH2-CO), 22.9(1C, CH_3); ms: m/z 616[M+1]. *Anal.* Calcd. for $C_{20}H_{11}Br_3N_2O_4S$. C, 39.05; H, 1.80; N, 4.45. Found: C, 39.15; H, 1.55; N, 4.40.

1-(3, 5-dinitro-phenyl) - 2 - (2-methyl-4-oxo-4H-thiochromene-8- carbonyl) pyrazolidine-3, 5-dione (5h): Orange crystal, yield 69.35%, melting range 245-247 °C; IR (KBr): 3075, 1760, 1685, 1540 and 1346 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm): 3.58 (s, 3H), 4.43 (s, 2H), 7.2-7.3 (d, 2H), 7.5 (d, 1H), 7.7-7.9 (m, 4H); ^{13}C NMR ($CDCl_3$, δ, ppm): 177.5, 172.0, 167.0, 164.9 (4C, C=O), 162.8, 150.3, 139.0, 136.7, 134.5, 132.4, 130.0, 127.8, 125.9, 123.7, 121.2, 119.1(14C, Ar-C), 40.6(1C, -CO-CH_2-CO), 20.8 (1C, CH_3); ms: m/z 469[M+1]. *Anal.* Calcd. for $C_{20}H_{12}N_4O_8S$. C, 51.28; H, 2.58; N, 11.96. Found: C, 51.35; H, 2.45; N, 11.85.

1-(2-methyl-4-oxo-4H-thiochromene-8-carbonyl) -2-(2, 4, 6- tri-iodo-phenyl) pyrazolidine-3, 5-dione (5i): Yellow crystal, yield 70.55%, melting range 325-328 °C; IR (KBr): 3075, 1760, 1720 and 1048 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm):3.64 (s, 3H), 4.65 (s, 2H), 6.9 (d, 1H), 7.2-7.3 (d, 1H), 7.4-7.5 (t, 3H), 7.6-7.8 (d, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 180.0, 174.8, 169.9, 167.4 (4C, C=O), 164.4, 157.7, 152.2, 144.8, 142.3, 140.2, 138.1, 136.0, 133.6, 131.5, 95.5, 91.2 (14C, Ar-C), 44.7(1C, -CO-CH_2-CO), 23.9 (1C, CH_3). *Anal.* Calcd. for $C_{20}H_{11}I_3N_2O_4S$. C, 31.77; H, 1.47; N, 3.71. Found: C, 31.35; H, 1.405; N, 3.65.

1-(4-hydroxyphenyl)-2-(2-methyl - 4- oxo - 4H-thiochromene – 8 - carbonyl) pyrazolidine - 3, 5-dione (5j): Colorless crystal, yield 67.45%, melting range 176-177 °C; IR (KBr): 3390, 3074, 1760, 1668 and 1248 cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ, ppm):3.4 (s, 3H), 4.62 (s, 2H), 6.92 (d, 2H), 7.1-7.2 (t, 2H), 7.56-7.64 (t, 2H), 7.8-8 (m, 2H), 9.7 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, ppm): 177.0, 171.8, 166.7, 164.2 (4C, C=O), 154.3, 150.0, 138.0, 135.9, 133.5, 131.6, 129.1, 127.0, 124.9, 122.3, 120.1, 117.9 (14C, Ar-C), 44.7(1C, -CO-CH_2-CO), 23.9 (1C, CH_3); ms: m/z 395[M+1]. *Anal.* Calcd. for $C_{20}H_{14}N_2O_5S$. C, 60.91; H, 3.58; N, 7.10. Found: C, 60.55; H, 3.35; N, 7.15.

Microbiological Activities:

Test microorganisms: Antimicrobial activity of newly synthesized compounds were screened against nine bacterial strains: *Bacillus subtilis* (MTCC 441), *Staphylococcus epidermidis* (MTCC 3615), *Micrococcus luteus* (MTCC 11948), *Staphylococcus aureus* (MTCC 11949), *Bacillus pumilis* (MTCC 2466), *Bacillus cereus* (MTCC 430); *Klebsiella pneumonia* (MTCC 4030), *Escherichia coli* (MTCC 1573), *Pseudomonas aeroginosa* (MTCC 4673) and three fungal strains:

Aspergillus niger (MTCC 478), *Candida albicans* (MTCC 7253), *Fusarium solani* (MTCC 4947) were selected.

Preparation of the Samples and Standard Solution: The synthesized compounds (5a-5j) were dissolved in 10% DMSO at the concentrations of 50, 100, 250, 500, 1000, 1250 and 1500 μg/mL, respectively. Ciprofloxacin and Clotrimazole, used as the standard drugs for antibacterial and antifungal studies, respectively, were also dissolved in 10% DMSO at the concentrations of 10μg/mL.

Method: Antimicrobial activity of the synthesized compounds (5a-5j) contains pyrazolidine-3,5-dione moiety was evaluated by cup-plate method. Nutrient broth suspension of test microbe (10 mL) was added to 100 mL of sterile molten nutrient agar growth media (cooled to 45°C), mixed well, and poured on to sterile petri plates. The agar was allowed to solidify and was then punched to make six wells/cups, using a 6 mm sterile cork borer (separate borer for each organism), to ensure proper distribution of wells in the periphery and one well in the center. Agar plugs were removed and 50 μL solutions of test samples (each compound in seven concentrations) was poured into the corresponding marked well using micropipette. Triplicate plates of each organism were prepared. The plates were left at room temperature for 2 hrs to allow diffusion of samples and then incubated face upward, at corresponding temperature of each organism, for 48 hrs [23]. The diameters of zone of inhibition were measured to the nearest millimeter (the cup size also included) and are presented in **Table 1**.

TABLE 1: MEANS DIAMETER OF ZONE OF INHIBITION (MM) OF SYNTHESIZED COMPOUNDS (5a-5j), STANDARD AND CONTROL AGAINST VARIOUS MICROORGANISMS

Sr. No.	Compounds	Conc.	Gram +ve strains						Gram –ve strains			Fungal strains		
		(μg/mL)	BS	SE	ML	SA	BP	BC	KP	EC	PA	AN	CA	FS
		50	7	-	8	7	-	8	7	-	5	-	-	7
		100	10	-	10	9	-	10	9	-	8	7	8	9
		250	14	8	13	13	8	13	11	8	11	9	10	11
1.	5a	500	16	10	16	15	10	15	14	11	14	12	13	13
		1000	18	12	19	18	14	17	17	14	18	14	14	15
		1250	20	13	21	20	16	19	21	16	19	15	16	17
		1500	21	15	22	22	18	21	23	18	21	17	18	19
		50	6	-	8	-	-	7	8	9	-	-	-	8
		100	8	7	9	-	-	10	10	11	-	-	8	10
		250	10	9	11	5	6	12	12	13	-	8	10	12
2.		500	14	11	13	8	9	14	14	15	6	10	12	14
	5b	1000	16	14	15	10	11	16	16	18	8	12	14	16
		1250	18	16	17	13	13	18	18	20	10	14	16	18
		1500	20	18	19	14	17	20	19	21	13	16	18	20
		50	-	-	-	7	-	5	6	-	7	-	-	-
		100	-	-	-	9	8	7	8	-	9	-	-	-
		250	8	-	7	11	9	9	10	8	10	7	-	5
3.	5c	500	10	9	10	14	11	11	14	10	11	9	6	7
		1000	12	12	13	16	13	13	16	12	12	11	8	9
		1250	14	15	14	18	15	15	18	15	14	13	10	10
		1500	16	17	16	20	19	17	20	17	17	15	14	12
		50	8	-	7	8	8	7	-	8	7	-	-	-
4.	5d	100	10	8	9	10	10	9	8	10	9	8	-	-
		250	13	10	11	12	12	11	10	12	11	10	-	5
		500	15	13	14	14	14	13	12	14	13	14	6	7
		1000	17	15	16	17	15	15	14	16	15	17	8	9
		1250	19	16	18	20	16	17	16	18	17	18	10	10
		1500	24	18	20	23	20	19	18	20	21	20	14	12
		50	7	-	7	6	8	8	-	8	8	7	-	-

		100	9	8	9	8	10	11	7	9	10	9	7	8
5.	5e	250	11	10	11	10	12	12	10	11	12	11	9	10
		500	13	12	13	13	14	14	12	13	13	13	10	12
		1000	14	14	15	15	16	17	14	15	15	15	12	14
		1250	16	16	17	17	17	18	16	17	17	17	14	16
		1500	18	17	19	19	19	20	18	19	20	19	16	18
		50	8	-	7	8	-	7	5	-	6	7	-	-
		100	10	-	9	10	-	10	8	7	8	9	8	-
		250	13	8	10	13	8	14	11	9	10	11	10	-
6.	5f	500	15	10	13	16	11	16	14	10	12	13	12	6
		1000	17	14	15	19	13	18	18	13	13	15	14	8
		1250	19	16	18	21	15	20	19	15	15	17	16	10
		1500	21	18	20	23	18	21	21	17	17	19	17	13
		50	7	8	8	8	7	-	8	7	-	-	-	-
		100	9	10	11	10	9	8	10	9	8	-	-	8
		250	11	13	13	13	11	10	12	11	10	7	8	10
7.	5g	500	13	15	16	15	13	12	14	13	12	10	11	13
		1000	15	17	17	17	15	14	16	15	13	12	12	15
		1250	17	19	18	19	17	16	19	18	15	14	14	16
		1500	21	20	20	22	19	19	21	20	17	16	15	18
		50	7	-	-	6	8	8	-	8	8	7	-	-
		100	9	8	9	8	10	10	7	9	10	9	7	8
8.	5h	250	11	10	11	10	12	12	10	11	12	11	9	10
		500	13	12	13	13	14	14	12	13	13	13	10	12
		1000	14	14	15	15	16	15	14	15	15	15	12	14
		1250	16	16	17	17	17	17	16	17	17	17	14	16
		1500	18	17	19	19	19	20	18	19	20	19	16	18
		50	8	-	6	-	-	7	8	9	-	-	-	8
		100	9	7	8	6	7	10	10	11	6	-	8	10
9.	5i	250	11	9	10	8	9	12	12	13	8	8	10	12
		500	13	11	14	10	11	14	14	15	9	10	12	14
		1000	15	14	16	12	13	16	16	18	11	12	14	16
		1250	17	16	18	13	15	18	18	20	13	14	16	18
		1500	19	18	20	16	17	20	19	22	14	16	18	20
		50	8	8	-	8	6	7	-	7	-	-	7	8
		100	10	9	7	10	8	9	8	9	8	7	9	10
10.	5j	250	12	11	10	12	10	11	10	11	10	9	11	12
		500	13	13	12	14	13	13	12	13	12	10	13	13
		1000	15	15	14	15	15	15	14	14	14	12	15	15
		1250	17	17	16	17	17	17	16	16	16	14	17	17
		1500	22	20	21	22	19	19	17	18	18	16	19	20
11.	Ciprofloxacin	10	26	24	30	24	27	25	27	28	27	-	-	-
12.	Clotrimazole	10	-	-	-	-	-	-	-	-	-	25	26	24
13.	Control	-	-	-	-	-	-	-	-	-	-	-	-	-

BS Bacillus subtilis, SE Staphylococcus epidermis, ML Micrococcus luteus, SA Staphylococcus aureus, BP Bacillus pumilus, BC Bacillus cereus, KP Klebsiella pneumonia, ES Escherichia coli, PA Pseudomonas aeroginosa, AN Aspergillus niger, CA Candida albicans, FS Fusarium solani

Control=10% v/v DMSO, (-) = no activity

Minimum Inhibitory Concentration: A series of glass tubes, containing different concentrations of the synthesized compounds (in 10% DMSO), with nutrient broth was inoculated with the required quantity of the inoculums to obtain a suspension of microorganisms which contained 10^5 colony forming units per milliliter.

One growth control tube was prepared with the addition of the compound and one blank tube was prepared without the addition of the microorganism. The tubes were incubated at 37 °C for 24 h. The turbidity produced in each tube was recorded on a UV-visible spectrometer. The observed MICs (μg/mL) are presented in **Table 2**.

TABLE 2: VALUES OF THE MINIMUM INHIBITORY CONCENTRATION OF THE SYNTHESIZED COMPOUNDS (5a-5j) AND REFERENCE STANDARDS

Sr. No.	Strain	MIC of Compounds (μg/mL)											
		5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	Cip	Clo
1.	*Bacillus subtilis*	30	70	90	30	110	80	90	120	110	50	04	-
2.	*Staphylococcus epidermis*	30	50	40	20	60	30	110	140	160	180	2.5	-
3.	*Micrococcus luteus*	110	90	70	60	140	150	170	200	250	190	3	-
4.	*Staphylococcus aureus*	140	160	200	140	250	220	150	120	130	140	3.5	-
5.	*Bacillus pumilus*	30	40	30	60	80	90	30	50	60	70	3	-
6.	*Bacillus cereus*	40	40	60	50	30	60	80	30	140	170	4.5	-
7.	*Klebsiella pneumonia*	120	100	80	100	140	150	60	180	190	150	2.5	-
8.	*Escherichia coli*	90	80	110	40	150	170	70	140	150	200	2	-
9.	*Pseudomonas aeroginosa*	200	140	160	130	150	180	70	140	210	250	3.5	-
10.	*Aspergillus niger*	200	140	170	70	250	100	80	70	40	80	-	1.5
11.	*Candida albicans*	250	250	150	250	200	180	160	140	200	180	-	2.5
12.	*Fusarium solani*	150	170	180	110	140	100	160	170	110	200	-	1.5

Cip Ciprofloxacin, *Clo* Clotrimaxole

RESULTS AND DISCUSSION: Ten novel compounds were synthesized by the fusion of two hetrocyclic moiety *i.e.* pyrazolidine-3, 5-dinones and thiochromene-4-one using starting material 2-mercapto benzoic acid. These compounds were characterized using IR, ^{1}H-NMR, ^{13}C-NMR, Mass Spectroscopy and Elemental Analysis. The IR spectrum of the synthesized compounds revealed the presence of C-H aromatic functional group at 3020-3080, C-N at 1227-1270, C=O at 1685-1764, C-H at 2930-3068 cm^{-1}. In ^{1}H-NMR spectra, δ values of the synthesized compounds were found in the range of 2.9-3.74 for alkyl protons and 6.96-8.3 for aromatic protons. ^{13}C-NMR spectra of the synthesized compounds were characterized various δ values. M^{+} and M+1 peak were observed in mass spectra of the synthesized compounds. Percentage of the carbon, hydrogen, and nitrogen in all the compounds was determined by microanalysis.

The compounds were screened for antimicrobial activity against six Gram-positive bacteria, three Gram-negative bacteria and three fungal strains. The minimum inhibitory concentrations (MICs) of all the active compounds were also determined by tube dilution method. All the compounds (5a-5j) were found more effective against Gram- negative strains than Gram- positive strains. The cell wall of Gram-negative bacteria is highly lipophilic character compare as Gram-positive bacteria. Therefore, the compounds show better activity against Gram- negative strains then Gram- positive strains. Compounds 5a exhibited good antibacterial activity, having 30μg/mL, against *Bacillus subtilus*, *Staphylococcus epidermis*, and *Bacillus pumilus*. Compound 5d was found to be most effective against *Staphylococcus epidermis* having lowest MIC (20 μg/mL) and good activity against *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus pumilus* and *Bacillus cereus* having MIC (30-60 μg/mL). Two bacterial strains (*Bacillus subtilis*, *Bacillus pumilus*) were found to be most sensitive against all the compounds at 30-60 μg/mL. *Staphylococcus aureus* was found to be the least sensitive strain against all the synthesized compounds. The antibacterial activity of the synthesized compounds was found to be better than antifungal activity. The antibacterial activity of the synthesized compounds was in the order of 5d<5a< 5g<5h<5f<5e<5b <5c<5j<5i. The antifungal activity was in the order of 5i<5h<5d<5g< 5j<5f<5b =5e<5c<5a.

CONCLUSION: The present research comprises the syntheses of some potential pyrazolidine analogs of 2-methyl-4-oxo-4H-thiochromene-8-carbonyl of pyrazolidine-3, 5-dinone and their antimicrobial potential. The compounds were screened for antimicrobial activity by cup-plate and tube-dilution methods. All the compounds exhibited more pronounced antibacterial activity than antifungal activity.

The electron withdrawing group at position-1 and electron withdrawing group at position-5 of the phenyl ring resulted in better activity. Also, substitution with iodine at position-1, 3 and 5 of phenyl ring resulted in better antifungal activity. These finding lead to the conclusion that the more active analogs were the more lipophilic ones, thereby suggesting that better permeation through the microbial cell wall could be the reason for this. Thus, lipophilic conjugates of pyrazolidine-3, 5 dione and thiochromene -4-one could be potential antimicrobial agents of the future.

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