Vol 14|Issue 2| 2024 |45-52.

ISSN 2249 - 7641
Print ISSN 2249 - 765X

International Journal of Pharmacology Research

www.ijprjournal.org

SYNTHESIS AND EVALUATION OF SOME NOVEL THIOCHROME DERIVATIVES AS ANTI-MICROBIAL AGENTS

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ABSTRACT

The substitution of electron releasing methyl or hydroxyl substituent either at 3rd or at 4th position of phenyl ring i.e. compound 23, 24 and 29 showed better antimicrobial activity while –NH2 substituent Containing Compound (compound 30) at 4th position of phenyl ring exhibited good activity. By comparing the antimicrobial activity of all the synthesized compounds, we conclude that electron withdrawing substituent's at 3rd, 4th and 5thposition of phenyl ring enhanced the activity while same substituent's at 2nd, 4th and 6th position resulted in slight decrease in activity. Presence of electron releasing substituent's at 3rd and 4th position of phenyl ring showed enhanced biological activity. While compared with the anti-inflammatory activity results, we conclude that the presence of electron releasing or withdrawing group at 4th position of phenyl ring resulted in an enhanced activity. Also, the presence of electron withdrawing group at 3rd or 5th or both positions of phenyl ring enhances the activity. Similarly, analgesic activity results showed that the electron withdrawing groups at 3rd , 4th and 5th position and electron releasing substituent's at 3rd and 4th position of phenyl ring enhances the analgesic activity.

Keywords: 1, 3, 5- triazine, Syntheses, Anti-microbial Agents, Pyrazolidine-3, 5-Dione.

INTRODUCTION

Heterocyclic chemistry is chemistry of those compounds that contains a hetero atom other than the carbon in a ring. The most common hetero atoms are nitrogen, oxygen and sulfur but other atom such as boron, phosphorus or silicon can also be members of heterocyclic rings.

Heterocyclic compounds are organic compounds containing at least one atom of carbon, and at least one element other than carbon, such as sulfur, oxygen or nitrogen within a ring structure. These structures may comprise either simple aromatic rings or non-aromatic rings. Some examples are pyridine (C_5H_5N), pyrimidine ($C_4H_4N_2$) and dioxane ($C_4H_8O_2$). Note that compounds such as cyclopropane, an anesthetic with explosive properties, and cyclohexane, a solvent, are not heterocyclic; they are cycloalkanes. The stem 'cyclic' implies a ring structure, whereas 'hetero' refers to an atom other than carbon, as above. Many heterocyclic compounds, including some amines, are carcinogenic.

1, 3, 5-Triaznes

1,3,5-Triazines are usually most easily obtained by substitution reaction on 2,4,6-triachloro-1,3,5-triazine, but the ring system can also be synthesized by cyclocondensation reactions.Trimerization of nitriles are imidates gives symmetrically substituted compounds; mono-substituted 1,3,5-triazine can be obtained via reaction of imidates with 1,3,5-triazine itself.12.

A route which allows the synthesis of 1, 3, 5-triazines with different substituents at each carbon is exemplified below –an N'-acyl-N, N-dimethylamidine with an amidine or guanidine to form a 1, 3, 5-triazine.13.

Antibacterial Agents

Waksman introduced the definition of antibacterial, "An Antibiotic or antibiotic substance produced from microorganism which has the capacity of inhibit or even destroying the other microorganism.

Laterally, advancement of new research of synthetic and natural chemistry necessitates inclusion of synthetic as well semi synthetic analogues. The miniature

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size and capacity for rapid multiplication of bacteria ensure that they are metabolically and structurally very different from mammalian cells and in theory there are several ways in which bacteria can be selectively killed or disabled. Antibacterial agents have been discovered designed rarely and those attack each of vulnerable sites that include bacterial cell wall, nucleic acid, ribosome, cell membrane and folate synthesis[1].

Antibiotic resistance has been approximately there for as long as antibiotics used to cure and prevention and treat of ailments. Certainly, the cause of antibiotic battle extends more rear in evolutionary term and reflects the shirking and defense of complex microbial flora in order to endure.

It is factual that early cure failure with antibiotics did not correspond to a important clinical difficulty since further classes of agents, with dissimilar cellular targets, were present there. It is the emergence of multiple resistances, mean to say resistance to numerous types of antibiotic agents that is cause major evils in the today clinical era.

Literature survey has already established potential activities of thiochrome nucleus. Our work on 2, 4, 6-trisubstituted -S- thiochrome derivatives and their evaluation for different biological activities**.**

MATERIALS & METHODS

The chemistry of the work deals with the synthesis of thiochromene derivatives. All the reagents used were of laboratory grade and were purchased from E Merck, Sigma-Aldrich, Loba Chemicals, and Ranbaxy Chemicals Ltd., India. Solvents used were purified as and when required. The melting points reported are uncorrected and determined in open capillary using Thiele's melting point apparatus. The reactions were monitored by thin layer chromatography. The purity of newly synthesized compounds was ascertained on the basis of chromatographic and physicochemical properties. Thin layer chromatography was performed on Silica gel G plates activated for 30 min. (120 ºC) and developed using different solvent systems. The spots were visualized by exposure to iodine vapour. Physicochemical and spectroscopic characterization of all synthesized compounds was done by assessment of their melting point, R_f values determined by TLC, Infra Red (FT-IR), ^{1}HNMR, ^{13}C NMR, Mass spectrum and elemental analysis.

IR spectra recorded using KBr discs on Shimadzu 8400-S FTIR. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DRX-300MHz and 100MHz spectrometer; chemical shifts are given in unit (ppm) relative to internal standard Tetramethylsilane (TMS) and using solvents $CDCl_3$ or DMSO-d_6 at CDRI, Lucknow. Mass spectra were recorded on VARIAN-500 mass spectrometer at IIT Powai, Mumbai.

Synthesis and physicochemical studies

Scheme I

Synthesis of 1-(2-Methyl-4-oxo-4*H*-thiochromene-8-carbonyl)-2-arylpyrazolidine-3, 5-dione (20 - 35) derivatives

Synthesis of Ethyl-2-mercaptobenzoate (2)

Ethyl-2-mercaptobenzoate (**2**) was prepared by esterification of 2-mercaptobenzoic acid following the reported method[60]. 2-Mercaptobenzoic acid (0.01 mol, 1.54 g) was dissolved in 80 mL of absolute ethanol and mixture was heated under reflux for 2 h. The reaction mixture was cooled in room temperature and conc. sulphuric acid (3.1 mL) was added. The reaction mixture was then refluxed for 1 h, the precipitate formed on addition of sulfuric acid gets dissolved during reflux.The mixture was then cooledto room temperature and the excess of sulphuric acid was neutralized by addition of sodiumbicarbonate (10 %). The solid thus produced was separated by filtration, dried and purified by recrystallization with ethanol.

Synthesis of Ethyl-2-methyl-4-oxo-4*H*-thiochromene-8-caboxylate[61] (3)

A solution of ethyl-2-mercaptobenzoate (**2,** 0.01 mol) and ethyl acetoacetate in a conical flask (21.85 mL), concentrated sulfuric acid was added. The mixture was stirred well and heated on a water bath at 75 – 80°C for 3 h. The reaction was cooled and poured in ice cold water. The solid thus formed was separated by filtration, dried and purified by recrystallization with ethanol.

Synthesis of 2-Methyl-4-oxo-*N*'-aryl-4*H*-thiochromene-8-carbohydrazide[62] (4 - 19)

A suspension of ethyl-2-methyl-4-oxo-4*H*-thiochromene-8-caboxylate (**3**, 0.01 mol) in methanol (10 mL) was prepared. The substituted phenyl hydrazine (0.01mol) was added to the suspension with constant stirring at room temperature. ethanol (20 mL) was added with slow stirring. The resulting solution was refluxed for 1-2 h and cooled at room temperature after completion of reflux. Finally the solid was separated by filtration and the solid was washed with diethyl ether (20 mL). The solid thus obtained was purified from appropriate solventto get respective carbohydrazides [4 – 19].

Synthesis of 1-(2-Methyl-4-oxo-4*H*-thiochromene-8-carbonyl)-2-arylpyrazolidine-3, 5-dione[63] [20 – 35]

A mixture of 2-Methyl-4-oxo-*N*'-aryl-4*H*-thiochromene-8-carbohydrazide (**4-19**) (0.01mol),diethyl malonate (0.015 mol), ethanol (60mL) and a drop of acetic acid was refluxed for 6-8 h. The reaction mixture was allowed to cool and kept overnight. The solid thus formed was filtered, dried and recrystallized from suitable solvents to get respective pyrazolidinedione derivatives [20 – 35].

BIOLOGICAL STUDIES

Antimicrobial activity (Cup-plate method[64])

A) *Antibacterial activity*

The antibacterial activity of the synthesized compounds was tested *in vitro* against *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 4698, *Staphylococcus aureus* ATCC 25923, *Staphylococcus hominis* ATCC 27844, *Bacillus pumilus* ATCC 14884, *Bacillus cereus* ATCC 11778, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 49565, *Salmonella typhi* ATCC 19430, *Klebsiella pneumonia* ATCC 13883, *Escherichia coli* ATCC 25922 and *Pseudomonas aeroginosa* ATCC 10145 by Cup-plate method.

B) *Antifungal activity*

The antifungal activity of synthesized compounds was performed similar to antibacterial activity against *Aspergillus niger* ATCC 9142, *Aspergillus awamori* ATCC 22342, *Candida albicans* ATCC 10231, *Alternaria alternate* ATCC 66868, *Microsporum canis* ATCC 11622, *Rhizoctonia solani* ATCC 76131, *Trichophyton longiformis* ATCC 22397, *Aspergillus flavus* ATCC 15517, *Fusarium solani* ATCC 38136 and *Trichoderma viride* ATCC 52440 by use of Potato dextrose broth as media for assay. The inoculated tubes were incubated for 48 hours.

The results of antimicrobial activity (zone of inhibition) of thiochromene derivatives are given in table number 3 and 4.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of all the synthesized derivatives was determined by Broth Dilution Method[65]. Nutrient broth and potato dextrose broth was procured from Himedia Laboratories. A set of sterilized test tubes with nutrient broth medium capped with cotton plugs were taken (1–12). The test compounds were dissolved in suitable solvent (DMF) and at the concentration of 600 μg mL^{-1}, which were serially diluted from 1 to 12. A fixed volume of 0.5 ml culture was added in all the test tubes and was incubated at 37 °C for 24 h. After 24 h, tubes were observed visually for turbidity. The results of antimicrobial activity (MIC) of thiocromene derivatives are given in table number 5 and 6.

Table 1: Antibacterial Activity of Thiochromene Derivatives (Cup plate method).

Compound	Name of microorganism (Zone of inhibition in mm)												
	P. v.	*B. s.*	*S. e.*	*P. m.*	*M. l.*	*S. a.*	*S. h.*	*B. p.*	*S. t.*	*K. p.*	*E. c.*	*B. c.*	*P.a.*
20	4.3	4.3	3.7	6.8	7.6	6.4	4.6	4.2	3.9	5.9	4.6	4.2	3.9
21	14.2	13.2	13.3	12.4	13.1	12.1	11.4	13.2	14.8	10.4	13.2	12.3	13.0
22	12.1	14.2	12.9	12.2	11.2	11.4	10.5	10.7	9.9	12.1	11.1	11.5	11.2
23	11.4	13.6	11.5	13.0	10.3	10.3	12.4	12.4	13.2	11.3	10.6	10.2	10.6
24	13.7	12.2	12.4	13.9	13.4	13.9	10.6	11.2	14.1	12.9	14.2	9.9	10.3
25	12.4	12.5	10.3	13.2	10.2	13.4	11.5	12.3	12.3	10.3	12.3	12.5	12.0
26	11.3	11.2	8.2	11.3	8.7	9.9	7.8	9.1	8.5	8.4	9.2	8.1	11.3
27	3.2	4.6	6.4	4.1	4.6	5.4	6.3	3.9	5.6	4.8	7.5	5.8	4.2
28	5.1	6.7	5.6	7.5	3.4	6.1	4.1	4.2	5.9	6.2	5.2	4.6	5.3
29	14.6	12.4	13.7	12.2	11.6	12.1	10.1	11.3	12.2	12.6	13.1	12.1	11.4
30	12.1	9.2	8.3	10.1	10.8	8.5	7.6	9.5	10.1	7.5	8.2	9.5	7.1
31	6.1	7.6	4.6	6.4	5.1	4.4	5.2	5.4	6.9	5.8	4.9	5.7	3.5
32	13.8	13.2	12.1	14.1	14.2	12.8	12.4	12.9	13.8	14.6	14.9	10.3	12.4
33	14.2	11.4	10.8	13.4	11.4	13.2	10.1	12.3	12.1	11.2	12.5	12.9	10.3
34	13.9	13.5	11.3	10.3	10.9	10.3	9.4	11.4	10.5	11.5	13.1	11.2	12.2
35	12.4	14.4	10.9	12.2	12.8	12.4	11.2	12.5	10.8	13.2	12.0	11.5	13.1
ciprofloxacin	16.2	15.7	14.5	15.5	15.1	15.8	13.4	14.6	16.1	15.3	16.3	15.4	15.6

P.v. – P. vulgaris, B. s. – B. subtilis, S. e. – S. epidermidis, P. m. – P. mirabilis, M. l.- M. luteus,S. a. – S. aureus, S. h. – S. hominis, B. p. – B. pumilus, S. t. – S. typhi, K. p. – K. pneumonia,E. c. –E. coli, B. c. – B. cereus, P. a. – P. aeroginosa

Table 2: Antifungal activity of thiochromene derivatives (Cup plate method).

Compound	Name of microorganism (Zone of inhibition in mm)									
	A.n.	*A. a.*	*C. a.*	*A.al.*	*M. c.*	*R. s.*	*T. l.*	*A. f.*	*F. s.*	*T. v.*
20	13.5	13.2	10.5	9.8	11.4	10.2	10.4	10.6	9.8	19.8
21	21.9	21.4	19.9	19.0	21.6	16.8	19.4	18.2	20.2	20.4
22	22.3	20.9	20.2	18.5	20.1	17.2	19.1	19.6	19.3	20.9

23	**22.1**	**19.8**	**19.6**	**18.8**	**19.4**	**16.5**	**18.7**	**19.2**	**19.8**	**19.7**
24	**21.2**	**20.5**	**20.8**	**19.2**	**22.1**	**17.8**	**19.4**	**18.9**	**19.5**	**21.3**
25	**20.8**	**19.8**	**20.2**	**19.6**	**22.6**	**20.3**	**17.6**	**20.1**	**20.4**	**17.2**
26	**19**	**16.2**	**13.6**	**15.1**	**15.7**	**13.6**	**13.9**	**14.3**	**14.6**	**16.6**
27	**17.8**	**15.3**	**14.9**	**16.2**	**13.9**	**14.1**	**14.7**	**14.4**	**14.2**	**10.1**
28	**13.8**	**14.8**	**11.4**	**12.7**	**12.2**	**10.5**	**11.1**	**11.7**	**10.8**	**20.6**
29	**21.3**	**19.3**	**18.7**	**19.4**	**20.3**	**19.4**	**16.9**	**20.3**	**19.9**	**16.2**
30	**18.6**	**15.1**	**16.2**	**15.9**	**14.2**	**13.9**	**14.2**	**14.4**	**14.5**	**9.8**
31	**15.1**	**11.7**	**12.6**	**10.3**	**9.9**	**12.2**	**10.8**	**10.2**	**10.1**	**22.0**
32	**22.4**	**21.2**	**20.9**	**18.7**	**22.8**	**18.6**	**17.7**	**18.7**	**17.9**	**20.1**
33	**21.6**	**19.3**	**18.8**	**18.5**	**22.3**	**20.3**	**19.9**	**19.5**	**20.6**	**19.9**
34	**20.9**	**20.6**	**19.1**	**18.1**	**20.6**	**20.9**	**19.2**	**20.1**	**20.5**	**20.3**
35	**21.2**	**19.7**	**19.4**	**18.9**	**21.2**	**19.8**	**18.7**	**19.9**	**19.6**	**19.8**
Clotrimazole	**24.3**	**22.8**	**22.1**	**20.6**	**23.7**	**21.4**	**20.1**	**22.4**	**21.6**	**23.2**

A.n. – A. niger, A. a. – A. awamori, C. a. – C. albicans, A. al. – A. alternate, M. c. – M. canis, T. l. – T. longiformis, A. f. – A. flavus, F. s. – F. solani, T. v. – T. viride.

Table 3: Antibacterial activity of thiochromene derivatives (Broth dilution method)

Compound	**Name of microorganism (MIC in µg/mL)**												
	P. v.	***B. s.***	***S. e.***	***P. m.***	***M. l.***	***S. a.***	***S. h.***	***B. p.***	***S. t.***	***K. p.***	***E. c.***	***B. c.***	***P.a.***
20	150	150	300	300	150	300	150	150	150	300	300	150	300
21	9.37	18.75	9.37	18.75	18.75	18.75	9.37	9.37	9.37	9.37	18.75	18.75	9.37
22	18.75	18.75	18.75	9.37	18.75	9.37	9.37	9.37	9.37	18.75	18.75	18.75	18.75
23	37.5	9.37	9.37	9.37	18.75	18.75	18.75	18.75	9.37	9.37	18.75	18.75	18.75
24	18.75	18.75	9.37	18.75	18.75	18.75	9.37	9.37	9.37	18.75	18.75	9.37	9.37
25	37.5	37.5	18.75	18.75	9.37	9.37	9.37	18.75	18.75	9.37	18.75	18.75	9.37
26	75	37.5	75	75	75	75	37.5	37.5	37.5	75	75	75	75
27	37.5	37.5	37.5	37.5	37.5	75	75	37.5	75	37.5	37.5	37.5	37.5
28	150	150	150	300	150	300	300	300	300	300	150	300	300
29	9.37	9.37	18.75	18.75	18.75	18.75	9.37	9.37	9.37	9.37	18.75	18.75	18.75
30	75	75	37.5	75	37.5	75	75	75	75	37.5	75	37.5	37.5
31	150	300	300	150	150	150	300	300	300	300	150	300	300
32	9.37	9.37	9.37	9.37	18.75	18.75	18.75	9.37	9.37	18.75	9.37	9.37	9.37
33	9.37	18.75	9.37	18.75	18.75	18.75	18.75	9.37	9.37	9.37	9.37	18.75	9.37
34	18.75	18.75	9.37	18.75	9.37	18.75	18.75	9.37	9.37	9.37	9.37	9.37	9.37
35	18.75	9.37	18.75	9.37	18.75	18.75	9.37	18.75	9.37	18.75	18.75	18.75	18.75
ciprofloxacin	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7

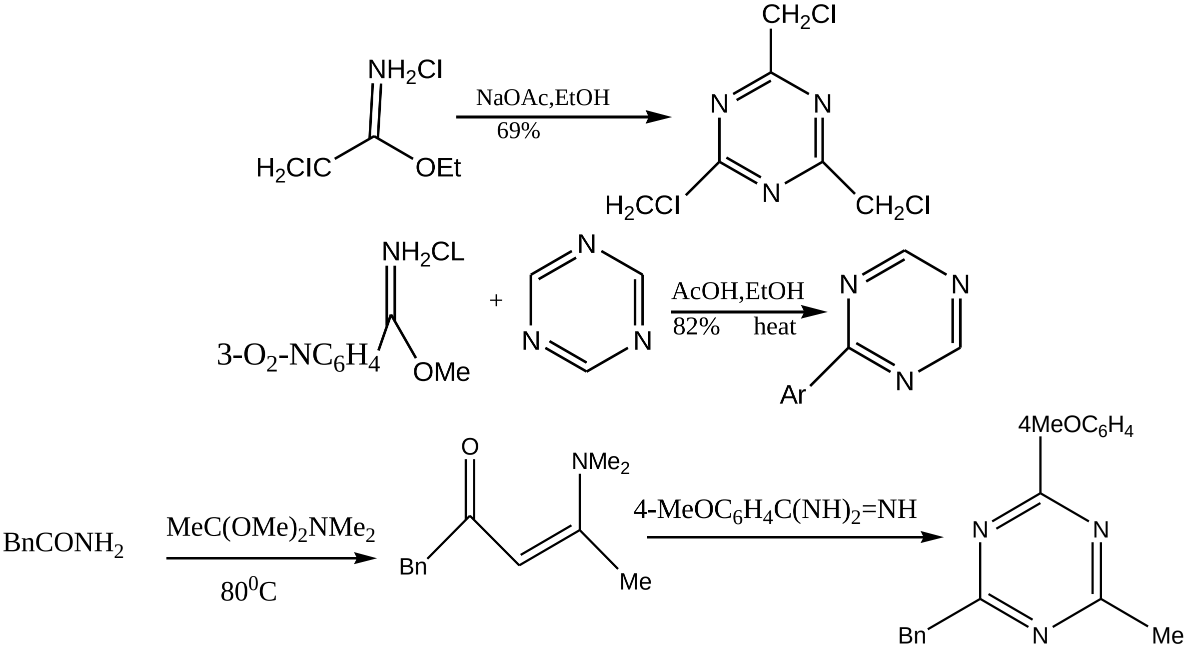
P.v. – P. vulgaris, B. s. – B. subtilis, S. e. – S. epidermidis, P. m. – P. mirabilis, M. l.- M. luteus,S. a. – S. aureus, S. h. – S. hominis, B. p. – B. pumilus, S. t. – S. typhi, K. p. – K. pneumonia, E. c. –E. coli, B. c. – B. cereus, P. a. – P. aeroginosa.

Table 4: Antifungal activity of thiochromene derivatives (Broth dilution method).

Comp. Code	**Name of microorganism (MIC's in µg/mL)**									
	A.n.	***A. a.***	***C. a.***	***A.al.***	***M. c.***	***R. s.***	***T. l.***	***A. f.***	***F. s.***	***T. v.***
20	150	150	75	75	75	150	150	150	150	150
21	4.68	4.68	4.68	9.37	9.37	9.37	4.68	4.68	4.68	4.68
22	4.68	9.37	9.37	4.68	9.37	4.68	4.68	4.68	4.68	4.68
23	4.68	4.68	4.68	9.37	9.37	9.37	4.68	9.37	4.68	4.68
24	9.37	9.37	9.37	9.37	9.37	4.68	4.68	9.37	4.68	4.68
25	4.68	9.37	9.37	4.68	4.68	9.37	4.68	9.37	9.37	9.37
26	37.5	37.5	18.75	37.5	37.5	18.75	18.75	18.75	37.5	37.5
27	18.75	37.5	37.5	18.75	37.5	18.75	37.5	18.75	18.75	18.75
28	150	150	75	150	75	150	150	150	150	150
29	4.68	4.68	4.68	9.37	9.37	9.37	4.68	9.37	4.68	4.68
30	37.5	37.5	18.75	18.75	37.5	18.75	18.75	37.5	37.5	37.5

31	75	150	75	150	150	150	150	150	75	150
32	4.68	4.68	4.68	4.68	4.68	4.68	4.68	4.68	9.37	4.68
33	9.37	9.37	4.68	4.68	4.68	9.37	4.68	4.68	9.37	4.68
34	4.68	4.68	4.68	9.37	9.37	9.37	4.68	4.68	4.68	4.68
35	4.68	4.68	4.68	4.68	9.37	9.37	9.37	4.68	9.37	9.37
clotrimazole	2.34	2.34	0.58	2.34	1.17	1.17	2.34	2.34	1.17	1.17

A.n. – A. niger, A. a. – A. awamori, C. a. – C. albicans, A. al. – A. alternate, M. c. – M. canis, T. l. – T. longiformis, A. f. – A. flavus, F. s. – F. solani, T. v. – T. viride.



Scheme I

Synthesis of 1-(2-Methyl-4-oxo-4H-thiochromene-8-carbonyl)-2-arylpyrazolidine-3, 5-dione (20 - 35) derivatives.

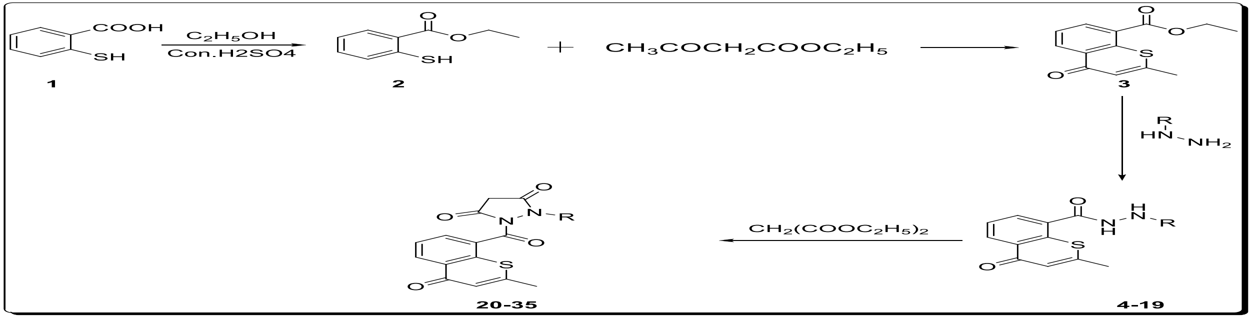


Figure 1: Reaction mechanism for thiochromene.

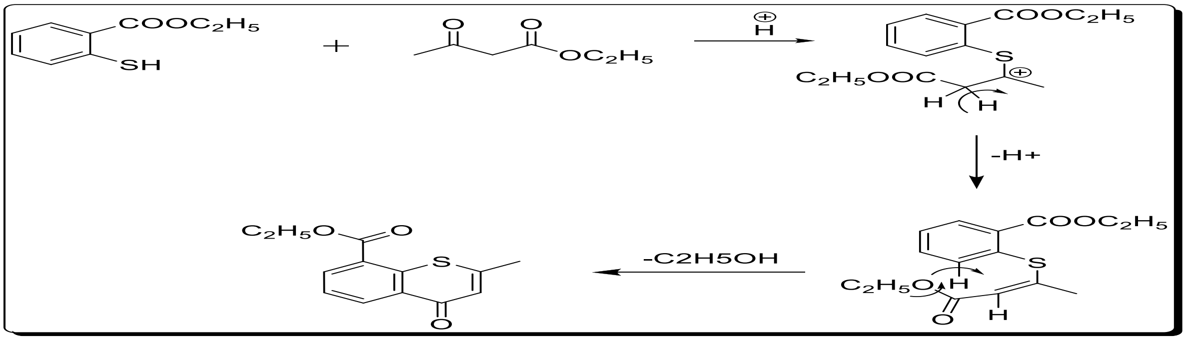
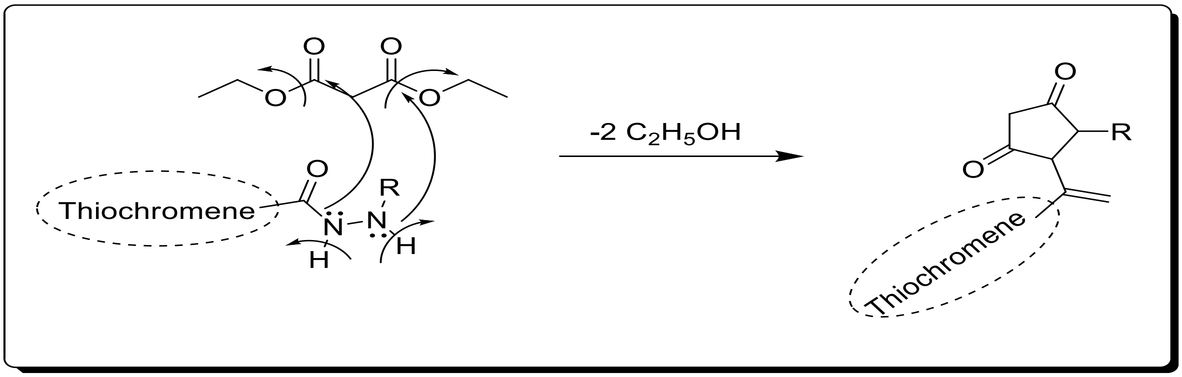


Figure 2: Reaction mechanism for pyrazolidinedione



RESULTS & DISCUSSION

The derivatives of thiochromene moiety exhibited antibacterial and antifungal activity and it is unexploited for the other biological activities. Also, the pyrazolidinedione derivatives exerted these activities along with anti-inflammatory and analgesic activity. In view of these facts, we decided to synthesize the novel pyrazolidinedione substituted thiochromene derivatives to get desired biological effect.

In the present work, novel **sixteen** titled derivatives of thiochromene were synthesized as outlined in Scheme I. The ethyl-2-mercaptobenzoate [2] was synthesized by reported procedure and the product was treated with ethyl-3-oxobutanoate, yielded ethyl-2-methyl-4-oxo-4*H*--8-carboxylate [3]. Subsequently the carbohydrazides [4-19] of [3] were obtained by reacting with substituted /unsubstituted phenylhydrazine. The title compounds [20-35] were obtained by cyclizing compounds [4-19] with diethyl malonate.

All the newly synthesized compounds were characterized by their physical properties and spectral data. The purity of newly synthesized compounds were confirmed by TLC. Spectral analysis (IR, ^{1}H, ^{13}C NMR and Mass spectrometry) of the compounds adequately supported the structures of the synthesized compounds.

The appearance of a band between 1764-1623 cm^{-1} (C=O) in the IR spectra; a singlet peak at δ value 4.3-5.0 for two protons of CH_2 of the pyrazolidinedione ring in the ^{1}H NMR spectra and a peak at δ 41.7-46.9 for carbon of CH_2 of the pyrazolidinedione ring supports the formation of pyrazolidinedione substituted thiochromene derivatives [20-35].

Molecular ion peaks of the synthesized pyrazolidinedione substituted thiochromene derivatives were obtained on the mass spectra, corresponds with their structures. These spectral data satisfactorily supports the formation of the title compounds.

The formation of thiochromene and pyrazolidinedione moieties is supported by the reaction mechanism as depicted in figure 67 and 68.

Biological Studies

Antimicrobial activity

Different *in vitro* methods are reported for evaluation of antimicrobial activity. The cup plate method and broth dilution method are employed widely for antimicrobial activity and MIC determination. Hence, these methods were considered for evaluation of newly synthesized compounds using ciprofloxazin and clotrimazole as reference drugs.

The results of antibacterial activity of the thiochromene derivatives showed better antibacterial activity against various bacterial strains. Compound 21 was active against *S. epiderimidisB. pumilus, P. vulgaris, S. hominis, S. typhi, K. pneumonia* and *P. aeroginosa*; compound 22 against *S. aureus, S. hominis, B. pumilus, P. mirabilis* and *S. typhi*;compound 23 against *B. subtilis, S. epidermidis, P. mirabilis, S. typhi*and *K. pneumonia*; compound 24 against *S. epiderimidis, S. hominis, B. pumilus, B. cereus, S. typhi,* and *P. aeroginosa*; compound 25 against *M. luteus, S. aureus, S. hominis, S. typhi,* and *P. aeroginosa*.

Compound 29 was activeagainst *B. subtilis, S. hominis, B. pumilus, P. vulgaris, S. typhi*and *K. pneumonia;* compound 32 against *B. subtilis, S. epiderimidis, B. pumilus, P. vulgaris, B. cereus, P. mirabilis, S. typhi, E. coli* and *P. aeroginosa*; compound 33 against *S. epiderimidis, B. pumilus, P. vulgaris, S. typhi, K. pneumonia, E. coli* and *P. aeroginosa*; compound 34 against *S. epiderimidis, Micro. luteus, B. pumilus, B. cereus, S. typhi, K. pneumonia, E. coli* and *P. aeroginosa* and compound 35 against *B. subtilis, S. hominis, P. mirabilis* and *S. typhi*

The results of antifungal activity of the thiochromene derivatives showed moderate to good antifungal activity against various fungal strains. Some of the thiochromene derivatives 21, 22, 23, 24, 25, 29, 32, 33, 34 and 35 exhibited good activity against *A. niger*, *A. Awamori,C. albicans*, *A. alternate*, *M. canis*, *R. solani*, *T. longiformis*, *A. flavus*, *F. solani,T. viride, A. flavus* and *A. fumigatus.*

The compounds 26, 27 and 30 showed good antibacterial as well as antifungal activity and compounds 20, 28 and 31were found to be inactive against all screened bacterial and fungal strains.

CONCLUSION

In conclusion, we conclude that pyrazolidinedione substituted thiochromene derivatives were synthesized satisfactorily and characterized by various spectral analyses. The compound having electron withdrawing substituent like chlorine at 3rdposition of phenyl ring i. e. compound 20 does not exhibit antibacterial as well as antifungal activity while introduction of electron withdrawing substituents like chlorine or fluorine Group at 3rd and 5th position of the phenyl ring i. e. compound 21 and 22 showed better antibacterial as well as antifungal activity. The change in the electron withdrawing substituent and its position i.e. replacement of chlorine or fluorine by bromine at 2nd, 4th and 6th position (compound 26) and by iodine (compound 28) at the same position of phenyl ring resulted in slight decrease in the activity and even devoid of activity respectively. The presence of electron withdrawing nitro substituents at 4th position of phenyl ring i.e. compound 32 showed better activity while compound 27 and 31 having same substituent at 3rd and/or 5th position showed slight decreased and loss of activity. The other electron withdrawing substituents i.e. –COOH (compound 25), -SO_3H (compound 33), -$COCH_3$ (compound 34) and -CHO (compound 35) showed good antimicrobial activity.

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