

SYNTHESIS CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF SOME PYRAZOLIDINEDIONE SUBSTITUTED THIOCHROMENE DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

Surbhi Tiwari*, Reetesh Yadav, Deepak Patel

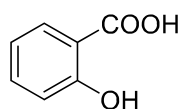
Abstract: All the newly synthesized compounds were characterized by their physical properties and spectral data. The purity of newly synthesized compounds was confirmed by TLC. Spectral analysis (IR, ^1H , ^{13}C NMR and Mass spectrometry) of the compounds adequately supported the structures of the synthesized compounds. The appearance of a band between $1764\text{-}1623\text{ cm}^{-1}$ ($\text{C}=\text{O}$) 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 ^1H 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.

Key words: Pyrazolidinedione Ring, Anti-Inflammatory, Synthesis, TLC, NMR.

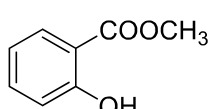
Introduction: Anti-Inflammatory, Antipyretic and Analgesic Agents: The various analgesic-antipyretic and anti-inflammatory drugs can be classified by three classes as which are as: -

Acidic Drugs: This includes seven subclasses as follows

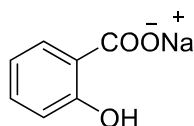
Examples:



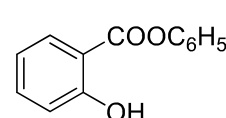
Salicylic acid



Methyl salicylate



Sodium salicylate



Phenyl salicylate

Salicylates: These are also known as salicylic acid derivative. Initially, salicylic acid was used in treatment for rheumatic fever and subsequently the other derivatives of salicylic acid were synthesized with antipyretic as well as analgesic and anti-inflammatory properties.

After discovery of novel drugs, a very prominent

***Corresponding author**

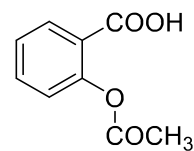
Shri Ram Institute of Pharmacy, Near ITI, Madhotal, Jabalpur

E-mail: surbhitiwari0603@gmail.com

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derivatives of salicylic acid was discovered called acetyl salicylic acid which was commonly known as aspirin. It is a mild analgesic and has been effectively used beside pain of low to moderate intensity.



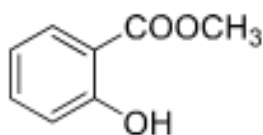
Aspirin

The irritating property of salicylic acid restricted it for using externally, to overcome all this; several derivatives of salicylic acid were prepared for its systemic use. These derivatives can be broadly classified into

a. Esters of salicylic acid:

The alkyl and aryl esters are used externally as counterirritants and had low analgesic property.

Example:



Methyl salicylate

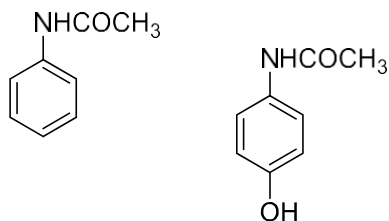
Various inorganic salicylates were made and used internally as analgesic agents. These include sodium salicylate, magnesium salicylate, sodium thiosalicylate, ammonium salicylate, choline salicylate, lithium salicylate and strontium salicylate. These salicylates have stomach irritating property.

b. Salicylate esters of organic acids:

The drug belongs to this class is Aspirin which is extensively used as antipyretic, analgesic and anti-inflammatory property. This has few unpleasant effects like allergic reactions (asthma) and urticaria and basically it has gastric irritating property.

Different salts of aspirin were prepared to minimize the undesirable side effects and to provoke analgesia better and faster than aspirin. The salts are calcium aspirin and aluminium aspirin.

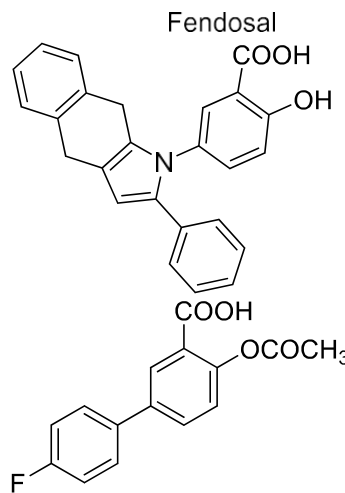
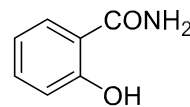
c. Salicylamide is a derivative of salicylic



acid and has quick onset and deeper analgesic effect than the aspirin.

Some other important derivatives of salicylic acid are benorylate, fendosal, diflunisal, salsalate and

flufenisal.



Flufenisal

ii. Para aminophenol derivatives

Para amino phenols have analgesic and antipyretic activity. Few of them are metabolites of aniline which possess the analgesic property. These are also known as “coal tar analgesics” & very few drugs are of interest like acetanilide, paracetamol and phenacetin. Acetanilide when gets metabolized to paracetamol & aniline thus exhibits its therapeutic action; but the high toxicity related with the acetanilide restricted its use. antipyrine is the parent drug of this category and its modification resulted in aminopyrine and dipyron. Antipyrine and aminopyrine have analgesic, antipyretic and antirheumatic activity. Dipyron and aminopyrine causes fatal agranulocytosis and hence has partial use.

Mechanism of action Non-Steroidal Anti-Inflammatory Agents

On basis of mechanism of action classified into following types: -

a. Reversible competitive inhibitors: -

Examples for this class are fatty acids, (i.e. prostaglandins) and LO pathway (leucotrienes).

Ibuprofen has a binding affinity for cyclooxygenase similar that of arachidonic while the planar hydrophobic group bind to the enzyme to prevent hydrogen abstraction at C-13. The existence of aryl halogen is supposed to increase this activity due to its lipophilicity. acid. The carbonyl function of aryl acidic NSAID's is said to resemble the terminal carboxyl of arachidonic acid closely related to the substrate which has a comparable affinity for the cyclooxygenase (CO) and lipooxygenase (LO) enzymes which are not changed to oxygenated inflammatory products of CO pathway Reversible noncompetitive inhibitors

The reversible noncompetitive inhibitors have anti-oxidant or radical trapping property. During inflammation, a continual presence by lipid peroxides induces a free radical reaction that sustains cyclooxygenase activity. This may be blocked by an addition of radical scavengers or antioxidants which works as reversible noncompetitive inhibitors. Paracetamol acts by the same mechanism.

b. Irreversible inhibitors

The irreversible inactivation of cyclooxygenase is made by aspirin through the transacetylation of the lysyl amino group in the enzyme which is important for activity. As salicylic acid by chemically unable of acylating the enzyme, the anti-inflammatory action by salicylic acid may depend more on other mechanisms such as inhibition of leukocyte emigration and lysosomal stabilization. Within the brain, aspirin like drugs block this release of prostaglandins in the brain, followed by peripheral vasodilation and increased sweating

resulted into considerable heat loss from body. This brings down by body temperature to its normal^{6,7}.

Microbial infections are now a day's more common than during the one half of the century. Although, number of diverse classes of antimicrobial agents are in use but their use is limited due to development of microbial resistance. Also the side effect and ineffectiveness of analgesics and anti-inflammatory agents highlighted the need for improvement of novel and potential antimicrobial, analgesic agents and anti-inflammatory.

Inflammation is a physiological and pathological response that activates immune and non-immune cells that protect host cells or organs from infectious species, including bacteria, viruses, and toxins, by eliminating pathogens and promoting tissue repair and recovery. In well-controlled inflammation, the initiation phase is involved in host defense by rapid and robust immune activation. The resolution phase curtails inflammation and restores tissue homeostasis when the danger signal is eliminated. Uncontrolled inflammation can cause chronic inflammatory diseases, such as arthritis, colitis, and asthma. These diseases are associated with severe tissue damage and an increased risk for the development of cardiovascular diseases, cancer, and osteoporosis.

Methodology

Experiment Protocol:

All the chemicals and solvents used in the synthesis of pyrazolidinone-3, 5-dione were purchased as LR grade from Rankem. Chem. Ltd., Mumbai and Sigma-Aldrich Chemical Co., Lancaster and were used directly without any further purification. Thin layer chromatography was used for monitor the progress of synthesis and yield formation. The thin layer chromatography of synthesized compounds carried out on 0.25 mm precoated plate of silica gel 60F₂₅₄, E. Merck, Darmstadt, Germany by using different solvent medium. Identification of spots was done under UV lamp and in Iodine chamber. Detection of spots under UV lamp was done at both short and long wavelength. The melting points were determined by open capillary method and were uncorrected.

Infrared spectra (ν_{\max} in cm^{-1}) of synthesized compounds were recorded on a Shimadzu FTIR-8400s, Perkin Elmer 881 in the range of 400-4000 cm^{-1} in potassium bromide.

Mass spectra were recorded on JEOL SX 102/DA-600 instrument using electron spray ionization (ESI) method and fast atomic bombarding (FAB) method.

^1H NMR spectra (ppm, δ) were recorded on Bruker ADVANCE DRX 300 MHz/200MHz spectrometer with TMS as the internal standard.

Elemental analyses were performed on ElementarVario EL III. Analysis of elements such as carbon, hydrogen, and nitrogen by same instruments.

Synthesis and physicochemical studies Scheme I

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

Structures of thiochromene derivatives.

Compound	R	Compound	R
20		28	
21		29	
22		30	
23		31	

24		32	
25		33	
26		34	
27		35	

Synthesis of Ethyl-2-mercaptobenzoate (2)

Ethyl-2-mercaptobenzoate (2) was prepared by esterification of 2-mercaptobenzoic acid following the reported method⁶⁰. 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 hrs; the precipitate formed on addition of sulfuric acid gets dissolved during reflux. The mixture was then cooled to room temperature and the excess of sulphuric acid was neutralized by addition of sodium bicarbonate (10 %). The solid thus produced was separated by filtration, dried and purified by recrystallization with ethanol.

Synthesis of Ethyl-2-methyl-4-oxo-4H-thiochromene-8-carboxylate⁶¹ (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-4H-thiochromene-8-carbohydrazide⁶² (4 - 19)

A suspension of ethyl-2-methyl-4-oxo-4H-thiochromene-8-carboxylate (**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 solvent to get respective carbohydrazides (**4 - 19**).

Synthesis of 1-(2-Methyl-4-oxo-4H-thiochromene-8-carbonyl)-2-arylpyrazolidine-3, 5-dione⁶³ (20 - 35)

A mixture of 2-Methyl-4-oxo-N'-aryl-4H-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

Anti-inflammatory activity of the compounds by Carrageenan-induced rat paws

Oedema method

The anti-inflammatory activity screening for the prepared compounds **20-35** was determined *in vivo* by the acute carrageenan induced paw oedema standard method in comparison to indomethacin as reference drug⁶⁶. The test is based on the pedal inflammation in rat paws induced by sub-plantar injection of 0.2 mL carrageenan (0.2 %) in suspension (5 % Sodium CMC) into the right hind paw of the rats (the tested compounds were dissolved in distilled water with sonication). Male adult albino rats (120-150 g) were divided into groups, each of four animals. The thickness of the rat paw was measured by a Vernier caliper (SMIEC, China) before and after 1 h of carrageenan injection

to detect the inflammation induced by carrageenan. Test compounds at doses of 10 mg/kg (body weight) were injected i.p. to nine groups of rat. Control group received the vehicle (5 % NaCMC); while reference group received Indomethacin at 10 mg/kg (body weight). The difference between the thicknesses of the two paws was taken as a measure of oedema. The measurement was carried out at 1, 2, 3, and 4 h after injection of the tested compounds, reference drug and the vehicle. The anti-inflammatory activity was expressed as percentage inhibition of oedema volume in treated animals in comparison with control group.

Percentage inhibition of oedema = $(V_c - V_t/V_c) \times 100$ where V_c and V_t are the volumes of oedema for the control and drug treated-animal groups, respectively.

Potency of the tested compounds was calculated relative to indomethacin "reference standard" treated group according to the following equation:

Analgesic activity (Acetic acid induced writhing in mice)

Acetic acid induced writhing method was adopted to evaluate analgesic activity⁶⁷. Writhing is defined as a stretch, tension to one side, lean-to of hind legs, contraction of abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response. Male albino mice weighing between 20-25 g were divided for study. The animals were divided into 5 groups (n = 6 in each groups). All animals received 0.1 ml acetic acid 0.6 % v/v i.p. and first group served as control. Group II served as positive control and received Diclofenac. The groups III, IV and V received 10 mg/kg of body weight of thiochromene derivatives 30 minutes prior to the administration of acetic acid i.p. The writhing effect was indicated by stretching of at least one hind limb. This was observed for 30 minutes and change in number of writhings in test group compared with standard treated and control treated group⁴³. The percentage inhibition was calculated by following,

Percentage inhibition = $1 - (N_t/N_c) \times 100$

Where N_t is average number of writhing in treated group and N_c is average number of writhing in control group.

Acute toxicity: The median lethal dose (LD_{50}) of the most active compound **23**, **25**, **27**, **29**, **31**, **33** and **35** was determined in mice. A group of male adult albino mice of five animals (25-30 g) was injected (i.p.) at a certain grade. The percentage of mortality was determined 72 hrs after injection. The computation of LD_{50} was processed by a graphical method.

Results and discussion: The derivatives of thiochromene moiety exhibited 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

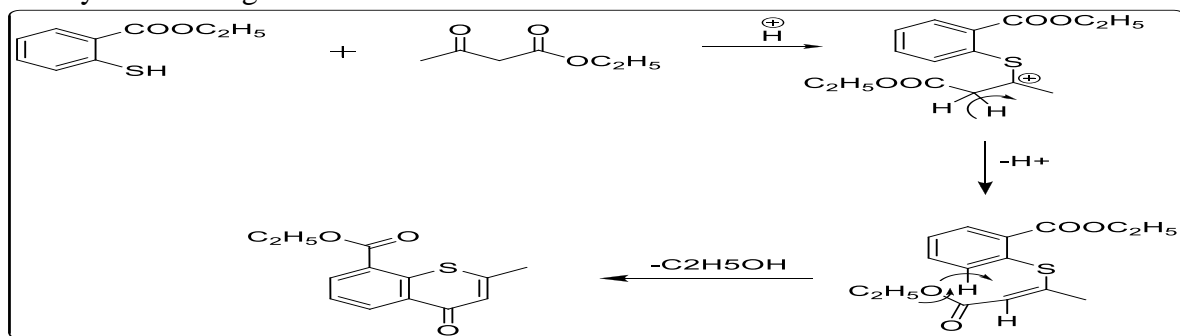
/unsubstituted phenylhydrazine. The title compounds (**20-35**) were obtained by cyclizing compounds (**4-19**) with diethyl malonate.

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

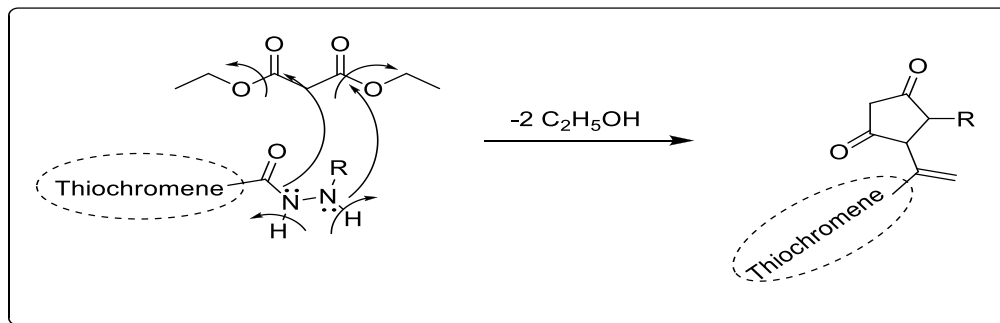
The appearance of a band between $1764-1623\text{ cm}^{-1}$ ($C=O$) in the IR spectra; a singlet peak at δ value 4.3-5.0 for two protons of CH_2 of the pyrazolidinedione ring in the 1H NMR spectra and a peak at δ 41.7-46.9 for carbon of CH_2 of the pyrazolidinedione ring supports the formation of pyrazolidinedione 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 8.1 and 8.2.



Reaction mechanism for thiochromene



Reaction mechanism for pyrazolidinedione

Biological Studies: Anti-inflammatory activity:

The anti-inflammatory activity of the target compounds **20-35** (at a dose of 10 mg/kg body weight) was determined *in vivo* by the acute carrageenan-induced paw oedema standard. The anti-inflammatory properties were recorded at successive time intervals 0.5, 1, 2, 3, and 4h and compared with that of indomethacin (at a dose of 10 mg/kg body weight), used as reference drug. From these results, it was noticed after 1 h that some of the tested compounds exhibit considerable anti-inflammatory properties (especially Compd. No. **23, 25, 27, 29, 31, 33** and **35**) which reveal remarkable activities, with potency (percentage oedema inhibition of the tested compounds relative to percentage oedema inhibition of indomethacin) of 0.72, 0.64, 0.56 and 0.72.

Analgesic activity: The analgesic activity of the synthesized compounds **20-35** (at a dose of 10 mg/kg body weight) was determined *in vivo* by acetic acid induced writhing method using diclofenac as reference standard. The results showed that compound **21** having dichloro substituent at 3rd and 5th position of phenyl ring showed 31.98 % inhibition. The compound **22** having chloro at 3rd and fluoro at 5th position of phenyl ring, compound **27** having dinitro group at 3rd and 5th position, compound **31** having nitro group at 3rd position and compound **35** having carbonyl group at 4th position of phenyl ring are found to be equi-potent with 48.27 % inhibition. Compound **24** having methyl group at 3rd position of phenyl ring exhibited 39.55 % inhibition while compound **30** having amino group at 4th position of phenyl ring exhibited

excellent activity among the screened compounds with 49.60 % inhibition.

Acute toxicity (LD50): The median lethal dose (LD50) of the most active compounds **21, 22, 23, 24, 25, 27, 29, 30, 31, 33** and **35** was determined in mice, according to reported procedures. The results showed that all the tested compounds were non-toxic at doses up to 200 mg / kg.

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 3rd position 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**), -SO₃H (compound **33**), -COCH₃ (compound **34**) and -CHO (compound **35**) showed good antimicrobial activity.

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 substituents at 3rd, 4th and 5th position of phenyl ring enhanced the activity while same substituents at 2nd, 4th and 6th position resulted in slight decrease in activity. Presence of electron releasing substituents 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 substituents at 3rd and 4th position of phenyl ring enhances the analgesic activity.

References

- JH Block, JM Beale. 2010. Anti-inflammatory analgesics. In: Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. Lippincott Williams and Wilkins. 12th ed.; 753-763.
- TL Lemke, DA Williams, V F Roche, SW Zito. 2008. Nonsteroidal anti-inflammatory drugs. In: Foye's Principles of Medicinal Chemistry. Lippincott Williams and Wilkins. 6th ed. 2008. 954-1003.
- CD Gahhutt, JD Hepworth, BM Heron. 1995. Reactions of Some 2*H*-Chromenes and 2*H*-Thiochromenes with Triazolinediones. *Tetrahedron*. 51(48):13277-13290.
- T Cohen, F Chen, T Kulinski. 1995. Reductive Cleavage and Ring Expansion of Thiochromane and Benzo dihydrothiophene. *Tetrahedron Letters*. 36(25): 4459-4462.
- O Bruno, S Schenone, A Ranise, F Bondavalli, W Filippelli, G Falcone. 1999. Anti-inflammatory agents: new series of *N*- substituted amino acid with complex pyrimidine structures endowed with antiphlogistic activity. *Il Farmaco*. 54: 95-100.
- E Rasolofonjatovo, B Treguier, O Provot, A Hamze, E Morvan, JD Brion. 2011. Palladium-catalyzed coupling of *N*-tosylhydrazones with ortho substituted aryl halides: synthesis of 4-arylchromenes and related heterocycle. *Tetrahedron Letters*. 52:1036-1040.
- KC Majumdar, A Bandyopadhyay, A Biswas. 2003. Regioselective synthesis of pentacycliheterocycles by sequential [3,3] sigmatropic rearrangement of 2-(4'-aryloxybut-2'-ynyl-mercapto) thiochromen-4-ones. *Tetrahedron*. 59:5289-5293.
- KM Dawood. 2005. Regio and stereoselective synthesis of bis-spiropyrazoline-5,3'-chroman(thiochroman)-4-one derivatives via bis-nitrilimines. *Tetrahedron*. 61: 5229-5233.
- RS Kenny, UC Mashelkar, DM Rane, DK Bezawada. 2006. Intramolecular electrophilic hydroarylation via Claisen rearrangement: synthesis of chromenes, heterothiochromenes and heterodihydrothiochromenes. *Tetrahedron*. 62:9280-9288.
- SL Cui, J Wang, YG Wang. 2008. Efficient synthesis of 2-imino-1,2-dihydroquinolines and 2-imino thiochromenes via copper-catalyzed domino reaction. *Tetrahedron*. 64: 487-492.
- VY Sosnovskikh, DV Sevenard, VS Moshkin, VO Iaroshenko, P Langer. 2010. Reactivity of 3-formyl- and 3- cyanothiochromones toward some *N*- and *C*-nucleophiles. Novel synthesis of 3-substituted 2-aminothiochromones. *Tetrahedron*. 66: 7322-7328.
- FM Moghaddam, M Kiamehr, MR Khodabakhshi, Z Mirjafary, S Fathi, H Saeidian. 2010. A new domino Knoevenagel- hetero-Diels Alder reaction: an efficient catalyst-free synthesis of novel thiochromone-annulated thiopyranocoumarin derivatives in aqueous medium. *Tetrahedron*. 66: 8615-8622.
- R Sugiura, R Kozaki S, Kitani, Y Goshio, H Tanimoto, Y Nishiyama. 2013. A novel thiochromone-type photolabile protecting group for carbonyl compound. *Tetrahedron*. 69: 3984-3990.
- M Jafarzadeh, K Amani, F Nikpour. 2005. Solvent-free and room temperature synthesis of thiochromans in the presence of a catalytic amount of tungstophosphoric acid. *Tetrahedron Letters*. 46: 7567-7569.

15. LG Voskressensky, EA Sokolova, AA Festa, AV Varlamov. 2013. A novel domino condensation–intramolecular nucleophilic cyclization approach towards annulated thiochromenes. *Tetrahedron Letters*. 54: 5172–5173.
16. G Eisenbrand, M Otteneder, W Tang. 2003. Synthesis of N-acetyl-S-(3-coumarinyl)-cysteine methyl ester Synthesis of N- acetyl-S-(3-coumarinyl)-cysteine methyl ester and HPLC analysis of urinary coumarin metabolite. *Toxicology*. 190: 249-258.
17. NS Reddy, MR Mallireddigari, S Cosenza, K Gumireddy, SC Bell, EP Reddy. 2004. Synthesis of new coumarin 3-(N-aryl) sulfonamides and their anticancer activity. *Bioorg Med Chem Letters*. 14: 4093–4097.
18. S Lee, K Sivakumar, W Shin, F Xie, Q Wang. 2006. Synthesis and anti-angiogenesis activity of coumarin derivative. *Bioorg Med Chem Letters*.; 16: 4596–4599.
19. A Beillerot, JR Domínguez, G Kirsch, D Bagrel. 2008. Synthesis and protective effects of coumarin derivative against oxidative stress induced by doxorubicin. *Bioorganic & Medicinal Chemistry Letters*. 18: 1102–1105.
20. KV Sashidhara, A Kumar, M Kumar, J Sarkar, S Sinha. 2010. Synthesis and in vitro evaluation of novel coumarin–chalcone hybrids as potential anticancer agents. *Bioorg Med Chem Letters*. 20: 7205–7211.
21. M Roussaki, CA Kontogiorgis, D Hadjipavlou-Litina, S Hamilakis, A Detsi. 2010. A novel synthesis of 3-aryl coumarins and evaluation of their antioxidant and lipoxygenase inhibitory activity. *Bioorg Med Chem Letters*. 20: 3889–3892.
22. KV Sashidhara, A Kumar, RP Dodda, NN Krishna, P Agarwal, K Srivastava, SK Puri. 2012. Coumarin–trioxane hybrids: Synthesis and evaluation as a new class of antimalarial scaffolds. *Bioorg Med Chem Letters*. 22: 3926–3930.
23. RK Upadhyay, P Kumar. 2009. A novel synthesis of coumarins employing triphenyl(a-carboxymethylene)- phosphoraneimidazolide as a C-2 synthon. *Tetrahedron Letters*. 50: 236–238.
24. Irgashev RA, Karmatsky AA, Slepukhin PA, Rusinov GL, Charushin VN. 2013. A convenient approach to the design and synthesis of indolo[3,2-c] coumarins via the microwave-assisted Cadogan reaction. *Tetrahedron Letters*. 54:5734– 5738.