EVALUATION OF ANTI-TUBERCULAR ACTIVITY OF SOME SYNTHESISED BENZ SPIRO-OXIRANE DERIVATIVES OF INDANE-1,3-DIONE

Karthik R[1], Jasmin Sajni R[1], Sasikumar S[1*], Kalyan S Betanabhatla[2], Christina AJM[2], Jagan Athimoolam[2], K Sundara Saravanan[3].

1. Division of Pharmaceutical Chemistry, KM College of Pharmacy, Uthangudi, Madurai, India.
2. Division of Pharmacology, KM College of Pharmacy, Uthangudi, Madurai, India.
3. Department of Pharmacognosy, Madurai Medical College, Madurai, India.

Summary

In the development of organic therapeutic agents, pharmaceutical scientists have explored numerous approaches in finding and developing organic compounds that are now available to us in dosage forms suitable for the treatment of our ills and often for the maintenance of our health. The present work deals with evaluation of anti-tubercular activity of various aldehyde derivatives synthesized by Knoevenagel condensation method and substitution in the second position of indane-1, 3-dione nucleus. The formation of Spiro-oxirane derivatives by reaction with alkaline hydrogen peroxide was also attempted. The synthesized derivatives were screened for anti-tubercular activity and the compounds demonstrated some remarkable features to be actively considered as anti-tubercular drugs.

Key Words: indane-1,3-dione derivatives, anti-tubercular activity, tuberculosis, Spiro-oxirane, Knoevenagel condensation.

Short title: Evaluation of anti-tubercular activity of indane-1,3-dione derivatives.

Introduction

Tuberculosis, one of the oldest diseases known to affect humans, is caused by bacteria belonging to the *Mycobacterium tuberculae* complex, an acid-fast aerobic bacillus. It is estimated that today one-third to one-half of the world population is infected with *Mycobacterium tuberculae* leading to approximately 6% of all death worldwide. Tuberculosis is the leading worldwide cause of mortality resulting from an infectious bacterial agent.

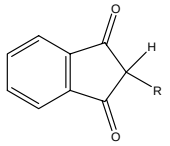
Mycobacterium tuberculae is transmitted primarily via the respiratory route. Tuberculosis is a disease, which mainly affects the lungs (80-85% of the case); although in up to one-third of cases other organs are involved. It is the most frequent cause of death worldwide due to single infectious agent, and in 1993 WHO declared Tuberculosis as a global public health emergency.

The physician is greatly challenged to provide optimal therapy for Mycobacterial illness because of the advent of AIDS, the increase in both drug-susceptible and multidrug-resistant tuberculosis, and the plethora of new antibiotics with antimycobacterial potential[1]. The aim of chemotherapy of tuberculosis is

1. To kill the dividing bacilli in the lung lesions.
2. To kill the persisters.

Pure organic compounds, natural and synthetic are the chief source of agents for the cure, the mitigation or the prevention of disease today. These agents have had their origin in a number of ways, *viz.*, (a) from naturally occurring materials of both plant and animal origin, (b) from the synthesis of organic compounds whose structure are closely related to those of naturally occurring compounds, and (c) that of pure synthetic has provided significant discoveries of medicinal chemistry [2].

1,3-indandiones have two ketone moieties. They are aromatic and not soluble in water. Derivatives of 1,3-indandione are represented by the general formula:



Our present study deals with the evaluation of anti-tubercular activity of the following synthetic derivatives of indane-1,3-dione:

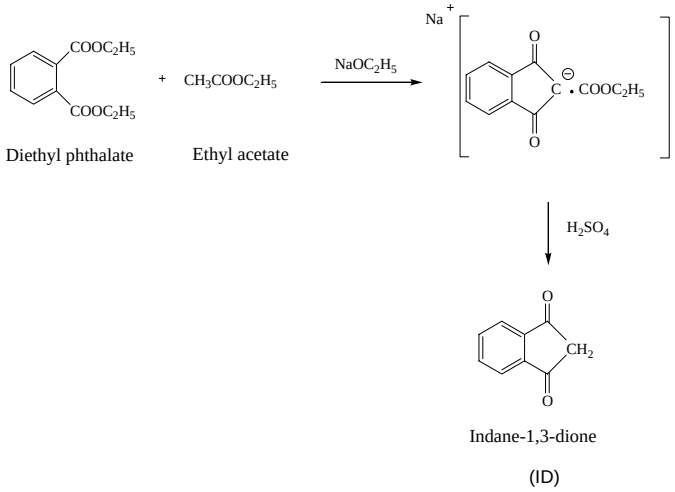
1.	Indane-1,3-dione	ID
2.	(1,3-diketone indane)-2-spiro-2´-[3´-(4-methoxy phenyl) oxirane].	ID_1
3.	(1,3-diketone indane)-2-spiro-2´-[3´-(4-chloro phenyl) oxirane].	ID_2
4.	(1,3-diketone indane)-2-spiro-2´-[3´-(4-fluoro phenyl) oxirane].	ID_3
5.	(1,3-diketone indane)-2-spiro-2´-[3´-(4-nitro phenyl) oxirane].	ID_4
6.	(1,3-diketone indane)-2-spiro-2´-[3´-(4-dimethylamino phenyl) oxirane].	ID_5
7.	(1,3-diketone indane)-2-spiro-2´-[3´-(2,3,4-trimethoxy phenyl) oxirane].	ID_6
8.	(1,3-diketone indane)-2-spiro-2´-[3´phenyl oxirane].	ID_7
9.	(1,3-diketone indane)-2-spiro-2´-[3´-(2-nitro phenyl) oxirane].	ID_8

Material and Methods

Experimental Methodology

Synthesis

1. The first step involves the synthesis of indane-1,3-dione by the reaction of diethyl phthalate and ethyl acetate in the presence of sodium wire and absolute ethanol[3].



Diethyl phthalate Ethyl acetate

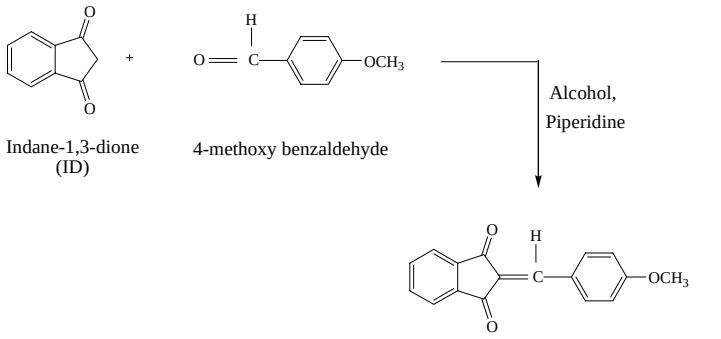
Indane-1,3-dione

(ID)

Melting point: 131ºc, Percentage yield: 51.67%

2. The second step involves indane-1,3-dione condensation with different aromatic aldehyde derivatives at C-2. (Knoevenagel condensation)[4]

Reaction of indane-1,3-dione with 4-methoxy benzaldehyde



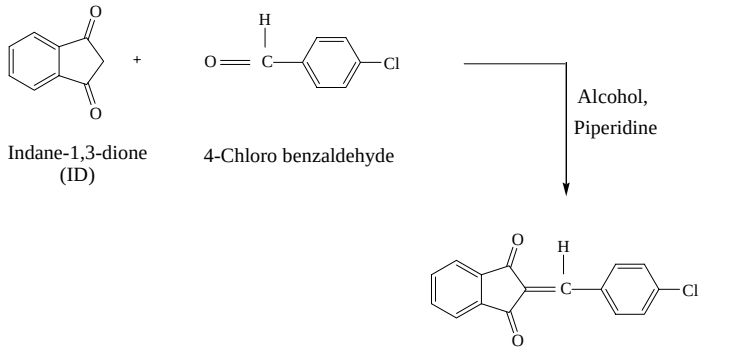
Indane-1,3-dione (ID)

4-methoxy benzaldehyde

2-(4-methoxy benzylidene)-indane-1, 3-dione

Melting point: 165ºc, Percentage yield: 87.1%

Reaction of indane-1,3-dione with 4-chloro benzaldehyde



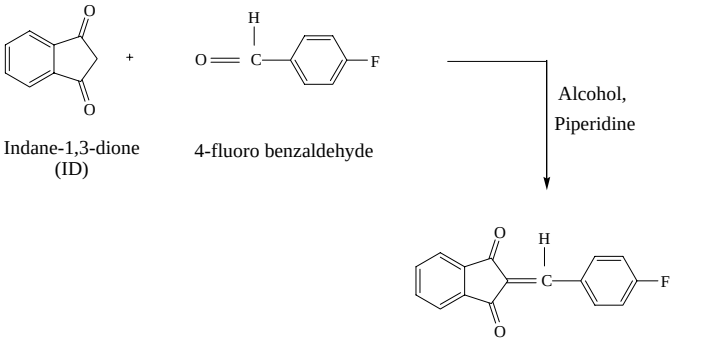
Indane-1,3-dione (ID)

4-Chloro benzaldehyde

2-(4-chloro benzylidene)-indane-1, 3-dione

Melting point: 175ºc, Percentage yield: 77.8%

Reaction of indane-1,3-dione with 4-fluoro benzaldehyde



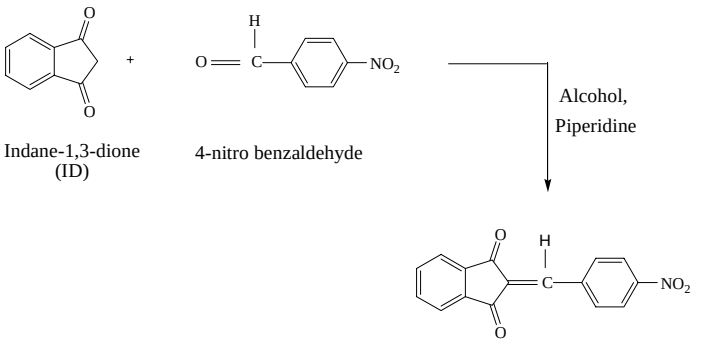
Indane-1,3-dione (ID)

4-fluoro benzaldehyde

2-(4-fluoro benzylidene)-indane-1, 3-dione

Melting point: 170ºc, Percentage yield: 87.3%

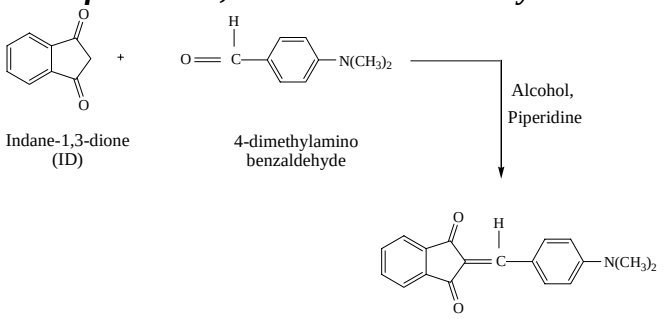
Reaction of indane-1,3-dione with 4-nitro benzaldehyde



Indane-1,3-dione (ID) + 4-nitro benzaldehyde → 2-(4-nitro benzylidene)-indane-1, 3-dione

Melting point: 225ºc, Percentage yield: 82.1%

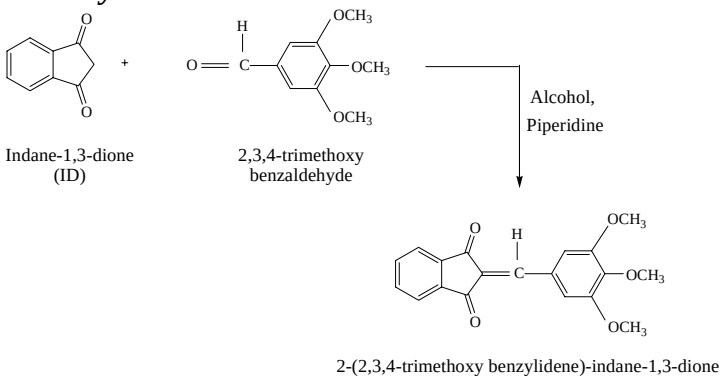
Reaction of indane-1,3-dione with 4-dimethyl benzaldehyde



Indane-1,3-dione (ID) + 4-dimethylamino benzaldehyde → 2-(4-dimethylamino benzylidene)-indane-1, 3-dione

Melting point: 180ºc, Percentage yield: 86.7%

Reaction of indane-1,3-dione with 2,3,4-trimethoxy benzaldehyde



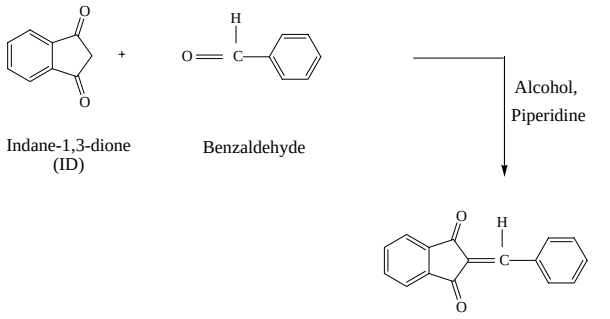
Indane-1,3-dione (ID)

2,3,4-trimethoxy benzaldehyde

2-(2,3,4-trimethoxy benzylidene)-indane-1,3-dione

Melting point: 185ºc, Percentage yield: 82.8%

Reaction of indane-1,3-dione with benzaldehyde



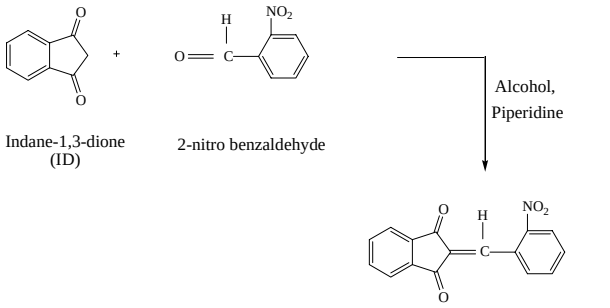
Indane-1,3-dione (ID)

Benzaldehyde

2-benzylidene-indane-1,3-dione

Melting point: 150ºc, Percentage yield: 89.7%

Reaction of indane-1,3-dione with 2-nitro benzaldehyde



Indane-1,3-dione (ID)

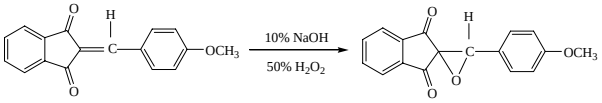
2-nitro benzaldehyde

2-(2-nitro benzylidene)-indane-1,3-dione

Melting point: 240ºc, Percentage yield: 93.4%

3. The third step involves the conversion of the above aldehyde derivatives into spiro oxirane derivatives in the presence of alkaline hydrogen peroxide[5].

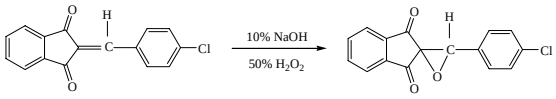
Formation of (1,3-diketone indane)-2-spiro-2´-[3´-(4-methoxy phenyl) oxirane]



2-(4-methoxy benzylidene)-indane-1, 3-dione ID_1

Melting point: 120ºc, Percentage yield: 94.33%

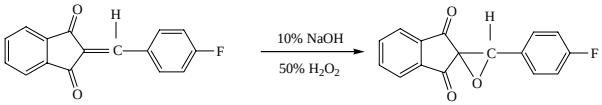
Formation of (1,3-diketone indane)-2-spiro-2´-[3´-(4-chloro phenyl) oxirane]



2-(4-chloro benzylidene)-indane-1, 3-dione ID_2

Melting point: 110ºc, Percentage yield: 71.42%

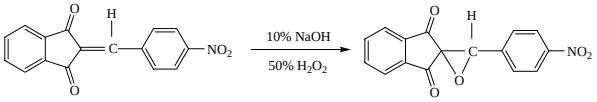
Formation of (1,3-diketone indane)-2-spiro-2´-[3´-(4-fluoro phenyl) oxirane]



2-(4-fluoro benzylidene)-indane-1, 3-dione ID_3

Melting point: 120ºc, Percentage yield: 67.30%

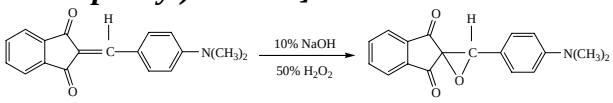
Formation of (1,3-diketone indane)-2-spiro-2´-[3´-(4-nitro phenyl) oxirane]



2-(4-nitro benzylidene)-indane-1, 3-dione ID_4

Melting point: 143ºc, Percentage yield: 95.24%

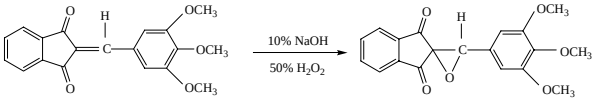
Formation of (1,3-diketone indane)-2-spiro-2´-[3´-(4-dimethylamino phenyl) oxirane]



2-(4-dimethylamino benzylidene)-indane-1, 3-dione ID_5

Melting point: 163ºc, Percentage yield: 85.71%

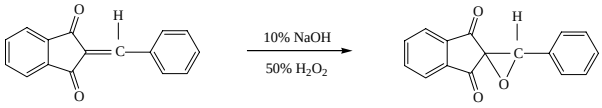
Formation of (1,3-diketone indane)-2-spiro-2´-[3´-(2,3,4-trimethoxy phenyl) oxirane]



2-(2,3,4-trimethoxy benzylidene)-indane-1,3-dione ID_6

Melting point: 165ºc, Percentage yield: 80.95%

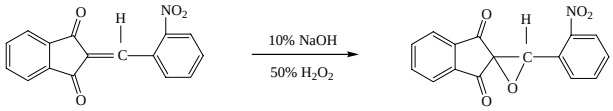
Formation of (1,3-diketone indane)-2-spiro-2´-[3´-phenyl oxirane]



2-benzylidene-indane-1,3-dione ID_7

Melting point: 135ºc, Percentage yield: 87.72%

Formation of (1,3-diketone indane)-2-spiro-2´-[3´-(2-nitro phenyl oxirane]



2-(2-nitro benzylidene)-indane-1,3-dione ID_8

Melting point: 152ºc, Percentage yield: 93.24%

The resulting spiro-oxirane derivatives of indane-1,3-dione were characterized by their chromatographic and spectroscopic properties. All the derivatives were screened for anti-tubercular activity.

ANTITUBERCULAR ACTIVITY[6, 7, 8]

Purpose and Rationale

The antitubercular activity of the various synthesized compounds was evaluated against *Mycobacterium tuberculae* H_{37} RV strain by Minimum Inhibitory Concentration (MIC) using Rifampicin as the reference standard.

Requirements

Mc cortney bottles (Sterilized)
Lowenstein- Jensen medium (sterilized)
Control - Ethanol
Standard - Rifampicin
Synthesized compounds - ID_2, ID_4, ID_5, ID_6, and ID_7.

Organism used

Mycobacterium tuberculae H_{37} RV strain.

Method

Minimum Inhibitory Concentration (MIC).

Composition of media

Lowenstein- Jensen Medium

1. Mineral salt solution
   Potassium dihydrogen phosphate (anhydrous) - 2.4gm
   Magnesium sulphate - 0.24gm
   Magnesium citrate - 0.6gm
   Asparagine - 3.6gm
   Glycerol - 12.0ml
   Distilled water - 600ml
2. Malachite green solution 2%
3. Egg solution

Procedure

1. All the ingredients of (1) were dissolved in 600ml of distilled water by heating. Autoclaved at 121º-126ºc for

20 minutes for sterilization. This solution was kept indefinitely and might be stored in suitable amounts.

2. 2% solution of Malachite green was prepared with sterile water with sterile precautions by placing the dye in the incubator for 2 hrs. This solution could be stored indefinitely and should be shaken before use.
3. The fresh eggs (not more than 4 days) were collected and washed carefully with soap, warm water and brush, rinsed in running water for 30minutes. The water was drained and the eggs were placed in a sterile tray, covered with a sterile paper and dried till next day. (Alternatively cleaning the shell with methylated spirit and burning it off dried eggs).
4. The hands were scrubbed with soap and water and dried with spirit. Then the eggs were cracked with a sterile knife and transfer the contents into a sterile beaker. The eggs were beaten with sterile eggbeater.
5. For complete medium mixed as follows.

   Mineral salt solution - 600ml
   Malachite green solution - 20ml
   Beaten egg (20-22 hen's egg) - 1000ml

   The complete medium was mixed and distributed as 10ml amounts in sterile screw capped tube or Mc Cortney bottles and the caps were screwed tightly on.

6. The tubes were placed in the slanting position in the water bath at 85ºc for 50minutes.
7. Then incubated at 37ºc for 48hrs for sterility checking.

Lowenstein- Jenson medium with Antibiotics-Ingredients

1. L.J. medium - as above.
2. Antibiotic - Rifampicin & synthesized compounds.

   a. Working solution was made from rehydarted antibiotic in ethanol as follows.

      Rifampicin - 1280µg/ml.
      Compounds - 1280µg/ml.

   b. Final concentration of antibiotics in L.J.medium

      Rifampicin - 5, 10, 20 and 40 (µg/ml).
      Compounds - 5, 10, 20 and 40 (µg/ml).

Preparation

1. Antibiotic stock solution

120mg of accurately weighed quantity of Rifampicin and the synthesized compounds (ID_2, ID_4, ID_5, ID_6 and ID_7) were dissolved individually with 30ml of ethanol to produce 4000µg/ml of stock solution. Then 6.4ml of stock solution was dissolved individually with 3.6ml of water to produce 2650µg/ml of working solution. Then 5ml of working solution was dissolved individually with 5ml of water to produce 1280µg/ml and preserved at 20ºc. Final concentration of 5,10,20 and 40(µg/ml) were prepared as given in the dilution chart. Finally 1ml of Rifampicin and synthesized compounds (ID_2, ID_4, ID_5, ID_6 and ID_7) were withdrawn and separately mixed with 9ml of L.J.medium using micropipette.

Procedure

Minimum Inhibitory Concentration method (MIC- method)

1. Antibiotics and synthesized compounds containing L.J.medium.
2. Dubos and Davis medium.

Composition

Bovine albumin solution 9%

Bovine albumin - 4.5ml
Water - 45.5ml
Mixed and sterilized by filtration.

Complete medium

Potassium dihydrogen phosphate	- 1gm
Disodium hydrogen phosphate	- 6.25gm
Sodium citrate	- 1.5gm
Magnesium sulphate	- 11.6gm
Tween- 80 (10% solution)	- 5ml
Casein hydrolysate (20% solution)	- 10ml
Distilled water	- 1145ml
Bovine albumin solution	- 40ml

Dissolved each salt separately in portions of the water then mixed these solutions into the tween and casein hydrolysate

dissolved in the remaining water. The medium should be at pH 7.2 distributed in culture containers, autoclaved at 115ºc for 10 minutes and added the Bovine albumin with sterile precautions.

3. Inoculam

Approximately 4mg moist weight of bacterial culture (Mycobacterium tuberculosis) was removed from a L.J. slope with 3mm external d.m (24 gauge wire) loop and transferred to Dubos and Davis medium to produce uniform suspension.

4. **Inoculation and Incubation**

One loop full of inoculam was inoculated on each slope using a 3mm external d.m (27 gauge wire) loop. Also inoculated as control on a drug free slope.

The standard sensitive H_{37}Rv strain as control was included with each batch. The tubes were incubated at 37ºc for 4 weeks.

Antimicrobial susceptibility testing of mycobacterium tuberculosis

Strain	: *Mycobacterium tuberculae* H_{37}RV
Medium	: L.J. medium
Incubation medium	: 4 weeks
Standard	: Rifampicin
Drug concentration	: 5,10,20 and 40 (μg/ml)

Table no: I

S.no	Compounds	Drug concentration (µg/ml)			
		5	10	20	40
1.	Standard	-	-	-	-
2.	ID_2	-	-	-	-
3.	ID_4	-	-	-	-
4.	ID_5	-	-	-	-
5.	ID_6	+	+	-	-
6.	ID_7	+	-	-	-

Control – (+)
(+) - indicates growth
(-) - indicates no growth

Results & Discussion

Among the several methods proposed for the synthesis of indane – 1, 3 – dione nucleus and subsequently some of its derivatives the methods involving condensation of phthalide and aromatic aldehyde was widely accepted. We attempted the condensation of phthalide and ethyl acetate, which resulted in good yields of indane-1,3- dione. This study defines the parameters consistent with synthetic convenience and higher conversions to indane – 1, 3 – dione. The parent nucleus indane – 1,3 – dione was condensed with various aldehydes and converted to yield their respective spiro-oxirane derivatives.

Characterization

The synthesized compounds were characterized by various methods, viz., (i) melting point, (ii) Nuclear Magnetic Resonance (NMR) spectrometry, (iii) Infrared spectrometry, and (iv) Mass spectrometry. The reports were in complete

agreement with their chemical structure. The purity of the compounds was further established by chromatographic method (TLC, Co-TLC).

Antitubercular activity

In order to ascertain their pharmaceutical application selective biological screening of these derivatives was carried out according to regular procedures and compared with standards. Our studies have demonstrated some remarkable features in the indane-1,3-dione derivatives to be actively considered as anti tubercular drugs. The need to design newer drugs for tuberculosis is clearly understood since the advent of HIV infections. Based on our modestly successful work, we propose to undertake QSAR and molecular modeling studies on these derivatives of indane-1,3-dione.

Acknowledgement

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***Corresponding author:**

Sasikumar S,
Division of Pharmaceutical Chemistry,
KM College of Pharmacy,
Uthangudi, Madurai – 625 107.
Tamil Nadu, India.
E-mail: rssasi@gmail.com
Phone: +91-9886762972.