IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Synthesis and Characterization of Isatin-Quinoline Hybrids as an Antitubercular Agents"

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Abstract:

Heterocyclic compounds can be used as therapeutic agents. Indole is an important class of heterocyclic compound containing nitrogen with wide variety of biological activities. Isatin is a derivative of indole which is indole-2, 3 Dione. Isatin is reported for anti- tubercular activity. Quinoline is also reported for various biological activities. So, a scheme was designed and isatin incorporated quinoline hybrids were prepared to improve biological activity. In the present research isatin incorporated quinoline **(7a, 7b, 7c and 7d)** were prepared, and were characterized by using TLC, IR, NMR and MASS spectral data. They were evaluated for anti-tubercular activity. Among those derivatives, compound **7a** and **7b** showed good activity.

Keywords: Isatin, Quinoline, Antitubercular, Conjugates.

1. Introduction:

Medicinal or pharmaceutical chemistry is a scientific discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. Pharmaceutical chemistry is focused on quality aspects of medicine and aims to assure fitness for the purpose of medicinal products. In practice, it includes understanding chemical characteristics of emerging active ingredients, followed by systematic, complete synthetic modification of new drugs to render drugs acceptable for therapeutic application. It encompasses the synthetic and computational attributes of studying existing medications and agents undergoing research in respect to their pharmacological activities i.e., comprehending structure–activity correlations.

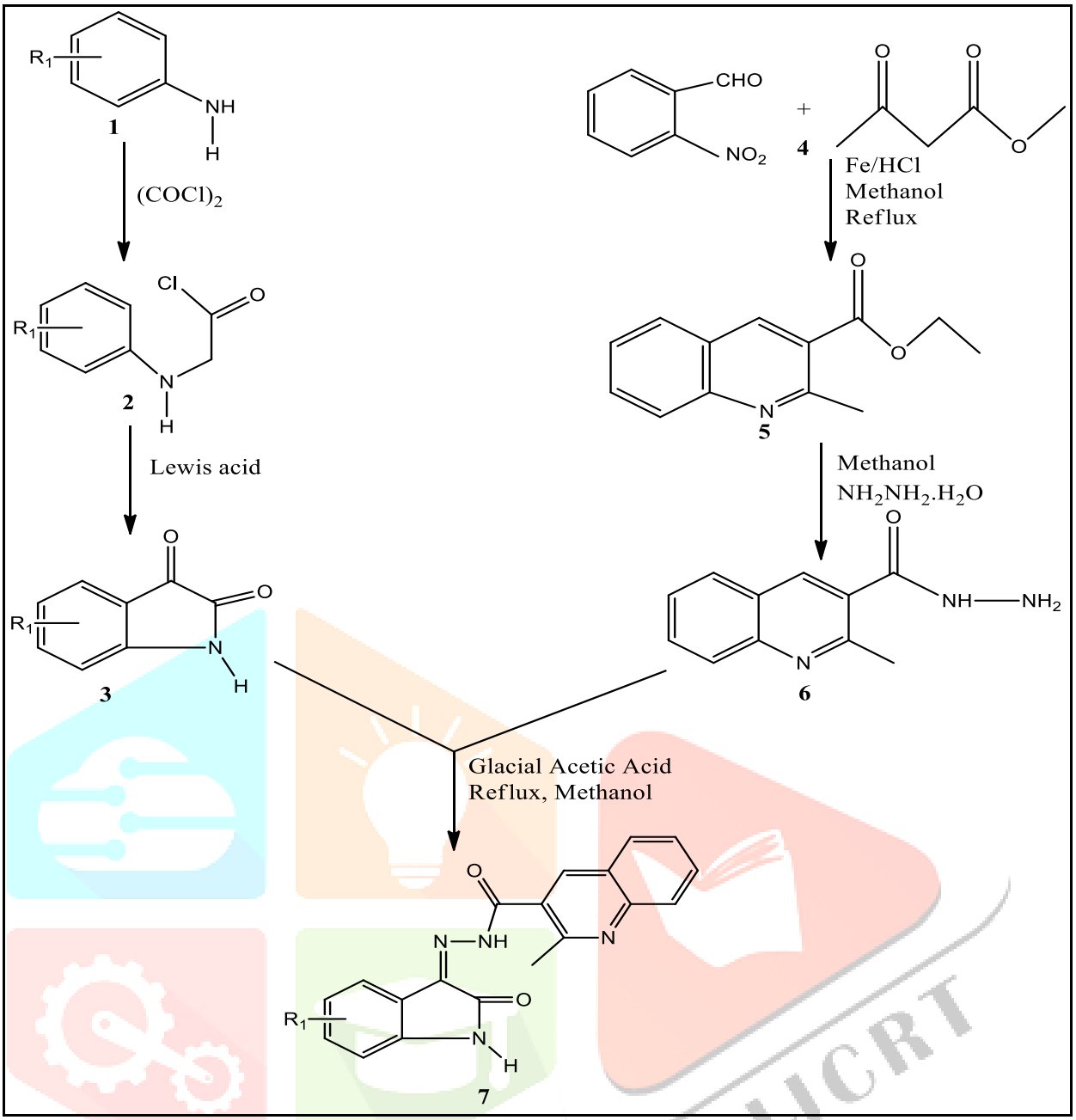
Heterocycles are cyclic organic molecules that contains minimum single heteroatom. Due to its reactivity in a variety of ailments, heteroatomic compounds are considered among the most important types of organic chemicals that are utilized in a number of different areas (1). Heterocycles are mostly employed in medicines, food crops, and veterinary medicine. They are also used as hand sanitizer, preservatives, protective coatings, co - polymers, and pigment materials. They are used in the organic synthesis as solvents. The heterocyclic ring is found in the primary framework of biological compounds including DNA and RNA, haemoglobin, vitamins, and others.

Isatin (1H-indoline-2,3-dione) is one of the indole derivatives which was prepared by oxidation of indigo (2). It comprises two cyclic rings, one of which is six-membered and the other is five-membered. In nature, isatin is found in plants of *Isatis genus* and in the species like *Melochia tomentosa* Aubl, *Couroupita guianensis* and *Boronella koniamboensis*, egg masses of Australian mollusc *Dicathais orbita* and in the parotid gland secretions of Bufo frog (3). Isatins have also been discovered in mammalian tissue, and their role as a biochemical process regulator has been the topic of interest for research purpose Both the rings are planar The derivatives of isatin exhibit diverse biological activities like antimicrobial (4), antibacterial (5), antimalarial (6), anti-tuberculosis(7), anti- inflammatory (8), anticancer (9), antihypertensive, anti-plasmodial (10), anti- HIV (11,12), etc.

Quinoline ring system consists of heterocycles in which benzene ring is fused with a pyridine ring. Quinoline has also been found to possess antimalarial, anticancer, antibacterial, anticonvulsant, cardio tonic, antifungal, anthelmintic, anti-inflammatory and analgesic activity (13). Quinoline backbone is found in a number of clinically relevant medications, demonstrating its considerable pharmacological importance (14).

Tuberculosis (TB) is a chronic bacterial infection, spread through the air, and caused by a bacterium called Mycobacterium tuberculosis (MTB) aerobic bacilli belonging to the Mycobacteriaceae, first identified in 1882 by Robert Koch, which can mainly attack the lungs, although can affect other organs as well. Currently TB is becoming again a worldwide problem and it was declared since 1993 by the World Health Organization (WHO), a global health emergency. The current problem of tuberculosis therapy is the emergence of multi-drug resistant (MDR) strains, caused by the improper use of antibiotics in chemotherapy of TB patients (15). Furthermore, the development of potent new antitubercular drugs without cross resistance with known antimycobacterial agents is urgently needed (16).

Bearing in mind the biological importance of the two molecular moieties, viz., isatin and quinoline, it was decided to study the condensation reactions of isatin with 2-methyl-quinoline 3-carbohydrazide (17). This series of compounds was screened for antitubercular activity.



R1= 4-Cl (a), R1= 4-Br (b), R1= H (c), R1= 5-NO2 (d)

Scheme 1. Schematic steps of 2-methyl-N'-[(3Z)-2-oxo-1, 2-dihydro-3H-indol-3-ylidine] quinoline-3-carbohydrazide derivatives.

2. Materials and Methods:

1.1. General

All chemicals used were of synthetic grade obtained from Lobachem and Kemphasol. Completion of the reaction was monitored by a thin layer chromatography (TLC) using Pre-coated Silica Gel250 Plates. Visualization was accomplished with ultraviolet light (256 nm). Melting points were determined on Analab melting point apparatus and were not corrected. All ^{1}H NMR spectra was recorded in DMSO-d_6 solvent. Chemical shifts are reported on Thermo SCIENTIFIC PicoSpin 80NMR relative to TMS internal standard on the δ (ppm) scale. The IR spectra were recorded on Bruker alpha ATR-IR. The mass spectra were recorded on LC-QTOF MS/MS.

1.2.Chemistry

Synthesis of isatins (indole-2, 3- diones) **(3)**

The different chlorooxalylanilides were synthesized from the respective anilines **(1)** on reaction with oxalyl chloride. Each chlorooxalylanilide **(2)** was subjected to cyclization using Lewis's acid aluminium chloride using Stolle's to yield the corresponding isatin **(3)**.

Procedure for the synthesis of ethyl 2-methyl-3-carboxylate (5)

Iron powder (4mmol) and 0.1M HCL (0.05mmol) were sequentially added to solution of an ortho-nitro benzaldehyde (0.302gm) in methanol. Stirred vigorously at 95^0C for 30 min. After that ß-keto ester (1ml) was added and reaction mixture was refluxed for 4 hrs. The resulting mass diluted with ethyl acetate and filtered. The filtrate was neutralized with saturated sodium bicarbonate solution, washed with water twice and extracted with ethyl acetate. The organic phase was dried with anhydrous Na_2SO_4 and solvent removed under reduced pressure. The crude material was recrystallized from hexane (Akbari et al. 2009).

Procedure for the synthesis of 2-methylquinoline-3-carbohydrazide (6)

Quinoline ester (**5**, 1eq) was dissolved in 5ml methanol and 3eq of 99% hydrazine hydrate was added. The resulting mixture was refluxed for 8hrs and cooled to room temp. The crystals were recrystallized using methanol (Abdel Aziz et al. 2013).

Procedure for the synthesis of 2-methyl-N'-[(3Z)-2-oxo-1, 2-dihydro-3H-indol-3-ylidine] quinoline-3-carbohydrazide (7)

To a solution of 2-methylquinoline-3-carbohydrazide (**6**, 0.01mol) and isatins (**3**, 0.01mol) in methanol, 6-8 drops of glacial acetic acid were added. The resulting reaction mixture was refluxed for 5hrs. The progress of the reaction was monitored by TLC. The residue was cooled, kept overnight and recrystallized using methanol (Gangarapu et al. 2014).

3. Spectral data of the synthesized compounds:

Ethyl 2-methylquinoline-3-carboxyate **(5)**

IR (ATR) cm-1: 3187 (ArH), 1724 (ester –C=O); 1H NMR (DMSO-d6) (ppm): 8.7 (1H, s), 8.1 (1H, d), 7.5 (1H, t), 1.6 (3H, t, CH3); LC_MS (ESI, m/z): 215 (M+H).

2-Methylquinoline-3-carbohydrazide **(6)**

IR (ATR) cm^{-1} d: 3241-3195 (-NH-NH2), 2983 (Ar-H), 1640 (-C=O); ^{1}HNMR (DMSO-d6) (ppm): (1H, s), 7.8 (1H, t), 7.63 (1H, s), 1.9 (3H, s, CH3); LC-MS (ESI, m/z): 202 (M+H).

N'-[(3Z)-4-chloro-2-oxo-1, 2-dihydro-3H-indol-3-ylidene]-2-methylquinoline-3- carbohydrazide **(7a)**

IR (ATR) cm^{-1}: 3282 (N-H), 2920 (Ar-H); ^{1H} NMR (ppm): 10.24 (1H, s, NH), 7.4 (8H), 1.93 (3H, s, CH3); LC-MS (ESI, m/z): 365 (M+1).

N'-[(3Z)-4-bromo-2-oxo-1, 2-dihydro-3H-indol-3-ylidene]-2-methylquinoline-3- carbohydrazide **(7b)**

IR (ATR) cm^{-1}: 3329 (N-H), 1748 (C=O); ^{1}H NMR (DMSO-d6) (ppm): 10.9 (1H, s-CONH), 10.06 (1H, s, NH), 2.1 (3H, s, CH3); LC-MS (ESI, m/z): 410 (M+1).

2-Methyl-N'-[(3Z)-2-oxo-1, 2-dihydro-3H-indol-3-ylidene] quinoline-3-carbohydrazide **(7c)**

IR (ATR) cm^{-1}: 3329 (N-H), 1748 (-C=O); ^{1}H NMR (DMSO-d6) (ppm): 11.39 (1H, s-CONH), 7.63(9H, m), 2.1 (3H, s, CH3); LC-MS (ESI, m/z): 330 (M+1)

N'-[(3Z)-5-nitro-2-oxo-1,2-dihydro-3H-indol-3-ylidene]-2-methylquinoline-3- carbohydrazide **(7d)**

IR (ATR) cm^{-1}: 3439 (N-H), 1725 (-C=O); ^{1}H NMR (DMSO-d6) (ppm): 11.04 (1H, s-CONH), 10.6 (1H, s, NH), 1.9 (3H, s, CH3); LC-MS (ESI, m/z): 376 (M+1).

4. Biological Activity:

Anti-tubercular activity

Assay procedure for determining Anti-tubercular activity by using Alamar Blue Dye assay:

i. The anti-mycobacterial activity of compounds was screened against Mycobacterium tuberculosis using Microplate Alamar Blue Assay (MABA) method.
ii. This assay is non-toxic and uses thermally stable chemicals and shows significant correlation with proportional and BACTEC radiometric method.
iii. To reduce evaporation of medium in the test wells during incubation, 200μl of sterile deionized water was injected to the outside perimeter wells of a sterile 96 well plate.
iv. The 96-well plate was filled with 100 μl of Middlebrook 7H9 broth, and the components were serially diluted on the plate.
v. Drug concentrations ranged from 100 to 0.2 g/ml.
vi. Parafilm was used to cover and seal the plates, which were then incubated at 37°C for five days.
vii. After 5 days, 25 litres of freshly made Alamar Blue reagent in a 1:1 ratio was added.
viii. The plate was then filled with 10% tween 80 and incubated for 24 hours.
ix. In the well, a blue tint indicated no bacterial growth, while a pink colour indicated bacterial growth.
x. The MIC was attributed to the lowest concentration of drug that did not cause the colour change from blue to pink.

Standard strain used: Mycobacterium tuberculosis (Vaccine strain, H37 RV strain):

ATCC No- 27294.

Standard values for the Anti-tubercular test which was performed:

Isoniazid – 1.6 μg/ml

Ethambutol – 1.6 μg/ml

Pyrazinamide – 3.125 μg/ml

Rifampicin – 0.8 μg/ml

Streptomycin – 0.8 μg/ml

5. Results and Discussion:

Table 1: The physicochemical characteristics of the newly synthesized compounds 7(a-d)

Compound	R	Mol. formula	Mol. Weight	Yield (%)	M.P (°C)
7a	4-Cl	$C_{19}H_{13}ClN_4O_2$	364	52	244
7b	4-Br	$C_{19}H_{13}BrN_4O_2$	409	54	222
7c	H	$C_{19}H_{14}N_4O_2$	330	59	258
7d	5-NO_2	$C_{19}H_{13}N_5O_4$	375	62	212

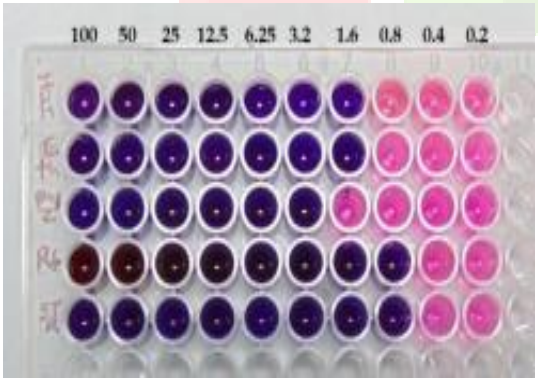


Fig. 1: Assay result of standard drugs

Fig. 2: Assay result of test drugs

Table 2: Anti tubercular activity of isatin-quinoline conjugates

Sr. No.	Sample	100 μg/ml	50 μg/ml	25 μg/ml	12.5 μg/ml	6.2 μg/ml	3.12 μg/ml	1.6 μg/ml
01	P1	S	S	S	S	S	S	S
02	P2	S	S	S	S	S	S	S
03	P3	S	S	S	R	R	R	R
04	P4	S	S	R	R	R	R	R

NOTE: S – Sensitive R – Resistant, Strain used: M tuberculosis (H37 RV strain): ATCC No- 27294

Chemistry

2-methyl-N'-[(3Z)-2-oxo-1, 2-dihydro-3H-indol-3-ylidine] quinoline-3-carbohydrazide conjugates were synthesized by simple and efficient methods as depicted in Scheme 1. Physical data of all synthesized compounds are shown in Table 1. The structure of all the newly synthesized compounds were confirmed by IR, ^{1}HMNR, and LCMS. The IR spectrum of compound **5** revealed the presence of Ar-H group due to the appearance of strong band at 3187 cm^{-1}, while that of –C=O of ester was observed at 1724 cm^{-1}. Further, in the ^{1}HNMR spectra 1.4 (triplet) ppm integrating for two and three protons, respectively. The LC-MS the signal derived from ester group (-OCH_2CH_3) was observed at 4.4 (quartet) and showed its molecular ion peak at 216 (M+H), which is in accordance with its molecular formula $C_{13}H_{13}NO_2$. The formation of hydrazide **6** from ester **5** was evidenced at 3241-3141 and 1640 cm^{-1} indicating the presence of -NH_2 and –C=O groups, by its IR, ^{1}HNMR, and LC-MS spectra. Its IR spectrum displayed absorbance bands respectively, while its ^{1}H NMR spectrum depicted disappearance of corresponding -OCH2CH3 peaks and appearance of 3.32 ppm (-NH-NH2) and 9.62 ppm (-NH-NH2) integrating for two protons and one proton, respectively (D2O molecular formula $C_{11}H_{11}N_3O$. The structures of compounds **7(a-d)** were interpreted of compound **6** showed a molecular ion peak at 202 (M+H), which matches with its exchangeable), confirming conversion of ester into hydrazide. The LC-MS spectrum of peak due to -NH_2, absence of carbonyl (C=O) peak around 1720 cm^{-1} demon- by its IR, ^{1}HNMR, and LC-MS spectra. Its IR Spectrum revealed that disappearance stated formation of Schiff bases.

6. Conclusion:

The 4 new isatin-quinoline hydrazone compounds (**7a-d**) were synthesized through multistep synthesis. The synthesized compounds were characterized by ATR-IR, 1H NMR, and LC-MS. The synthesized compounds were screened for in-vitro anti-tubercular activity. Among the tested compounds, 4-substituted compounds (**7c** and **7d**) exhibited effective potency in inhibiting the growth of Mycobacterium tuberculosis. The activity of compounds suggested that the introduction of electron donating group on forth position of the isatin ring significantly improved the activity.

7. Acknowledgements:

The authors of this research article are thankful to the Principal, Government College of Pharmacy, Karad for allowing us to present this paper. We are also thankful to the AICTE for providing research fellowship to complete our research work.

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