

AN EFFICIENT THREE COMPONENT ONE-POT SYNTHESIS OF -1,2,3,4-TETRAHYDRO-4-OXO-6-(5-SUBSTITUTED 2-PHENYL-1H-INDOL-3-YL)-2-THIOXOPYRIMIDINE-5-CARBONITRILE AS ANTIMICROBIAL AND ANTITUBERCULAR AGENTS

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ABSTRACT

Objective: The objective of the study was to synthesis of 1,2,3,4-tetrahydro-4-oxo-6-(5-substituted 2-phenyl-1H-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile derivatives (4a-c).

Methods: The structures of all these unknown compounds have been confirmed with the help of physical and spectral techniques such as IR, ¹H, and ¹³C NMR and mass spectral data and these newly synthesized compounds were evaluated for *in-vitro* antimicrobial and antitubercular activities.

Results: Screening studies have demonstrated that the newly synthesized compound 4a exhibited promising antimicrobial and antitubercular properties.

Conclusion: The final results revealed that compound 4a exhibited promising antimicrobial and antitubercular properties when compared to the standard drugs.

Keywords: Indole, Thioxopyrimidine, Antimicrobial, Antitubercular activities.

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INTRODUCTION

Heterocyclic compounds have been proved to be the most useful ones due to their possible practical applications. Heterocyclic compounds exhibits wide range of biological activities such as antibacterial and antifungal activities [1-6]. One-pot multi-component organic reactions (MCORs) are important and attractive due to the formation of multi-bonds in one pot, high atom economy, mild and simplified conditions, facile execution, and generation of complex product from a single operation process to available starting materials. MCORs are now being tuned for synthesizing various heterocyclic compounds due to their diverse biological activities [7,8].

The indole derivative has been reported to possess a wide variety of biological activities, namely, anti-inflammatory [9,10], anticonvulsant [11], cardiovascular [12], antibacterial [13], cyclooxygenase-2 inhibitor [14], and antiviral [15]. The significant contribution of many derivatives of indole in the development of medicinal chemistry is well recognized. Serotonin, known for its vasoconstrictor principle [16], plays a vital role as a neurotransmitter and in psychosis. Pyridine is used as a pioneer for pharmaceuticals, agrochemicals, and it is used as organic solvent and reagent. It plays a key role in mobilize both chemical and biological systems. Compounds containing fused pyrimidine ring has attracted much attention of researcher due to their wide range of biological activities, particularly in cancer and virus research [16]. Furthermore, substituted pyrimidine at position-2 or -4 with an amino group is known as pharmacophores in several structure-based drug design approaches in medicinal chemistry [17].

In addition to this, various analogs of pyrimidines have been found to possess antibacterial [18], antifungal [19], antileishmanial [20], anti-inflammatory [21], and analgesic [22] activities. Many thienopyrimidines are found to exhibit a variety of biological activities, including anti-inflammatory [23], antimicrobial [24], and analgesic [25]

properties. In view of the above observations and in continuation of our research work on the synthesis of biologically active molecules [26-33]. In the present investigation, we planned to synthesise of indole and pyrimidine nucleus embedded in one frame (indolyl-pyrimidine) to get a more potent molecule and testing for anti-microbial and anti-cancer activities.

METHODS

All the reagents were obtained commercially and used by further purification using standard procedures. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin-layer chromatography (TLC) using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (Spectrum ONE) Fourier-transform infrared (FT-IR) Spectrometer. The ¹H and ¹³C NMR (dimethyl sulfoxide [DMSO]-d₆) spectra were recorded with a BRUKER NMR 500 and 125 MHz spectrometers, and the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by electron impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer.

General procedure for the synthesis of (5-Substituted 2-phenyl-1H-indol-3-carboxaldehydes 1a-c) was prepared by the following literature method [34].

General procedure for the synthesis of 1,2,3,4-tetrahydro-4-oxo-6-(5-substituted 2-phenyl-1H-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile (4a-c): Appropriate mixture of 5-substituted 2-phenyl indole-3-carboxaldehydes (1a-c) (0.01 mole), ethyl cyanoacetate 2 (0.01 mole), and thiourea 3 (0.01 mole) in ethanol (25 mL) containing and potassium carbonate (0.01 mole) was taken in round bottom flask, refluxed on water bath for 7–8 h. The reaction completion of was monitored by TLC.

Then, the reaction mixture was poured into ice-cold water and acidified with acetic acid; then, the precipitate occurs which was filtered, washed with water, dried, and recrystallized with ethanol was to afforded 4a-c.

1,2,3,4-tetrahydro-4-oxo-6-(5-chloro 2-phenyl-1H-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile (4a): Yield: 89%; M.p.: 229–30°C; R_f 0.91 ethyl acetate: pet ether (1:1) mixture; FT-IR (KBr) (cm^{-1}): 3340 (CO-NH-CS), 3319 (NH-CS) 3101 (indole-NH), 2170 (CN), 1665 (CO), 1102 (CS); $^1\text{H NMR}$ (DMSO-d_6): δ : 12.01 (s, 1H, indole NH), 10.11 (s, 1H, CO-NH-CS), 9.96 (s, 1H, NH-CS), 7.30-7.71 (m, 8H, Ar-H); $^{13}\text{C NMR}$ (DMSO-d_6): 174.3, 169.8, 164.4, 133.9, 133.8, 128.8, 128.5, 127.1, 126.6, 123.3, 121.3, 119.5, 115.9, 112.8, 104 and 80.6; MS (EI) m/z 378.034 (M^+), 380.036 ($M^+ + 2$); Anal. Calcd. for $\text{C}_{19}\text{H}_{11}\text{N}_4\text{OSCl}$ (380.036), C, 60.24, H, 2.93, N, 14.19. Found: C, 60.31, H, 2.90, N, 14.16%.

1,2,3,4-tetrahydro-4-oxo-6-(5-methyl 2-phenyl-1H-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile (4b): Yield: 86%; M.p.: 205–06°C; R_f 0.86 ethyl acetate: pet ether (1:1) mixture; FT-IR (KBr) (cm^{-1}): 3348 (CO-NH-CS), 3325 (NH-CS) 3102 (indole-NH), 2170 (CN), 1659 (CO), 1108 (CS); $^1\text{H NMR}$ (DMSO-d_6): δ : 12.08 (s, 1H, indole NH), 10.08 (s, 1H, CO-NH-CS), 10.01 (s, 1H, NH-CS), 7.26-7.61 (m, 8H, Ar-H); 2.47 (s, 3H, CH_3); $^{13}\text{C NMR}$ (DMSO-d_6): 174.1, 169.6, 164.3, 133.8, 133.4, 128.5, 128.2, 127.0, 126.8, 123.5, 121.5, 119.6, 116.1, 112.2, 105, 80.6 and 23.6; MS (EI) m/z 358 (M^+); Anal. Calcd. for $\text{C}_{20}\text{H}_{14}\text{N}_4\text{OS}$ (358.089), C, 67.02, H, 3.94, N, 15.63. Found: C, 67.03, H, 3.92, N, 15.68%.

1,2,3,4-tetrahydro-4-oxo-6-(2-phenyl-1H-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile (4c): Yield: 88%; M.p.: 196–97°C; R_f 0.90 ethyl acetate: pet ether (1:1) mixture; FT-IR (KBr) (cm^{-1}): 3350 (CO-NH-CS), 3327 (NH-CS) 3106 (indole-NH), 2179 (CN), 1662 (CO), 1105 (CS); $^1\text{H NMR}$ (DMSO-d_6): δ : 12.05 (s, 1H, indole NH), 10.09 (s, 1H, CO-NH-CS), 10.01 (s, 1H, NH-CS), 7.31-7.70 (m, 9H, Ar-H); $^{13}\text{C NMR}$ (DMSO-d_6): 175.2, 169.2, 165.2, 134.8, 133.1, 129.1, 128.1, 127.2, 126.5, 123.1, 121.4, 119.5, 116.4, 112.3, 105.1, 80.6; MS (EI) m/z 344 (M^+); Anal. Calcd. for $\text{C}_{19}\text{H}_{12}\text{N}_4\text{OS}$ (344.073), C, 66.26, H, 3.51, N, 16.27. Found: C, 66.30, H, 3.48, N, 16.22%.

In-vitro antimicrobial activity

The *in-vitro* antimicrobial activity of all the synthesized compounds (4a-c) was carried out by broth microdilution method [35] in dimethylformamide at concentrations 500, 250, 125, and 62.5 $\mu\text{g/mL}$. Mueller–Hinton broth was used as a nutrient medium to growth and dilutes the compound suspension for the test bacteria and Sabouraud Dextrose broth used for fungal nutrition. Inoculum size for test strain was adjusted to 10^8 colony-forming unit per milliliter by comparing the turbidity. The strain employed for the activity was procured from the Department of Biotechnology, Sahyadri Science College, Shimoga.

The compounds (4a-c) were screened for their antibacterial activity against *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368), and *Pseudomonas aeruginosa* (MTCC-1688), as well antifungal activity against *Aspergillus oryzae* (MTCC-3567¹), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973), and *Aspergillus terreus* (MTCC-1782). DMSO used as a vehicle to get the desired concentration of compounds to test upon microbial strains. The lowest concentration which showed no visible growth after spot subculture was considered as minimum inhibitory

concentration (MIC) for each compound. The standard antibiotic used for comparison in the present study was gentamycin for evaluating for antibacterial activity and fluconazole for antifungal activity. The protocols are summarized in (Table 1).

Antitubercular activity using Alamar Blue Dye

The antitubercular activity of compounds (4a-c) was assessed against *Mycobacterium tuberculosis* H37R_v strain using a microplate Alamar blue dye assay [36]. Briefly, 200 μL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 μL of the middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100–0.2 $\mu\text{g/mL}$ and compared with standards pyrazinamide 3.125 $\mu\text{g/mL}$ and streptomycin 6.25 $\mu\text{g/mL}$. Plates were covered and sealed with parafilm and incubated at 37°C for 5 days. After this time, 25 μL freshly prepared 1:1 mixture of Alamar blue reagent and 10% tween-80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. The results are shown in Table 2.

RESULTS AND DISCUSSION

Chemistry

In the present study, indolyl-pyrimidine derivatives were synthesized using the conventional method. A rapid, improved, and eco-friendly synthesis of thiopyrimidines is carried out through a one-pot multicomponent reaction of 5-chloro 2-phenyl indole-3-carboxaldehyde 1a, ethyl cyanoacetate 2, and thiourea 3 in the presence of ethanolic K_2CO_3 using the conventional method to give 1,2,3,4-tetrahydro-4-oxo-6-(5-chloro 2-phenyl-1H-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile 4a. The compound 4a IR spectrum absorption peaks at 3340 and 3319 cm^{-1} which corresponding to the asymmetric stretching of 2-NH group of pyrimidine, the peak appeared at 3101 cm^{-1} which corresponding to the indole-NH, the sharp absorption peak appeared at 2170 cm^{-1} which corresponding to the nitrile function and the peak appeared at 1662 cm^{-1} due to the carbonyl group of pyrimidine nucleus. The IR absorption band at 1108 cm^{-1} which corresponds to the C=S stretching supports the formation of compound 4a. $^1\text{H-NMR}$ spectrum data showed that the signal appeared as a singlet at δ 11.10 which correspond to the indole NH, the two singlet appeared δ 10.08 and 10.01, ppm due to the two-NH protons of thiopyrimidine, respectively. The aromatic protons resonated as multiplet around δ 7.30–7.71 ppm, these proton NMR data support the formation of compound 4a. Mass spectral data show that the isotopic peaks at m/z 378.034 (M^+) and 380.036 ($M^+ + 2$), which clearly confirm the formation of compound 4a (Scheme 1). Similarly, other compounds 4b and 4c in the series were confirmed. The results were given in the material and method section.

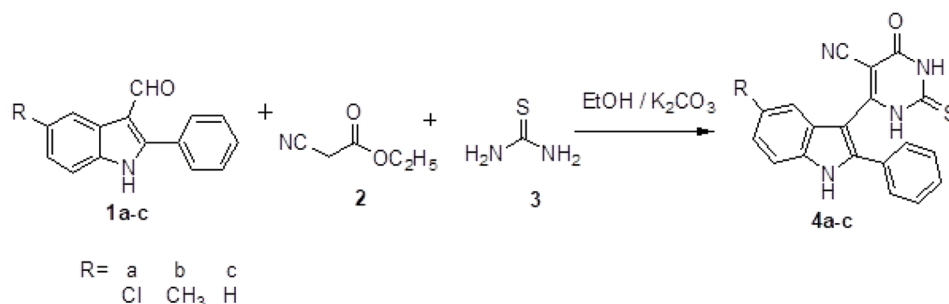
Antimicrobial activity

Antimicrobial activity results (Table 1) the MIC value it is clear that the tested compounds were active in the concentration range of 62.5–250 $\mu\text{g/mL}$ which is comparatively more or equipotent than the standards gentamycin and fluconazole. Antibacterial activity of

Table 1: *In-vitro* antimicrobial activities of compounds (4a-c)

Comp. code	Antibacterial activity (MIC $\mu\text{g/mL}$)				Antifungal activity (MIC $\mu\text{g/mL}$)			
	EC ^a	SA ^b	KP ^c	PA ^d	AO ^e	AN ^f	AF ^g	AT ^h
4a	62.5	125	125	125	125	62.5	125	250
4b	250	250	250	250	500	500	250	500
4c	125	500	500	500	500	250	125	500
Gentamycin	125	125	250	125	--	--	--	--
Fluconazole	--	--	--	--	125	62.5	125	250

^aEC- *Escherichia coli* (MTCC-723), ^bSA- *Staphylococcus aureus* (ATCC-29513), ^cKP- *Klebsiella pneumonia* (NCTC-13368), ^dPA- *Pseudomonas aeruginosa* (MTCC-1688), ^eAO- *Aspergillus oryzae* (MTCC-3567¹), ^fAN- *Aspergillus niger* (MTCC-281), ^gAF- *Aspergillus flavus* (MTCC-1973), ^hAT- *Aspergillus terreus* (MTCC-1782)



Scheme 1: Schematic pathways for synthesis of compounds (4a-c)

Table 2: Antitubercular activity of compounds (4a-c)

Comp. No.	MIC ^a values (µg/mL)
4a	3.125
4b	25
4c	12.5
Pyrazinamide	3.125
Streptomycin	6.25

MIC: Minimum inhibitory concentration

screened samples, compound 4a showed potent activity (62.5 µg/mL) against *E. coli* (MTCC-723).

Antifungal activity screening results revealed that the compound 4a showed potent activity (62.5 µg/mL) against *A. niger* (MTCC-281) and (125 µg/mL) *K. pneumonia* (NCTC-13368), this potent activity may be due to the presence of chlorine atom at C-5 position of indole system. Compound 4a exhibited equipotent activity against *S. aureus* (ATCC-29513) and *P. aeruginosa* (MTCC-1688).

Antibacterial study revealed that the compound 4a exhibited equipotent activity against all tested bacteria *A. oryzae* (MTCC-3567⁺), *A. niger* (MTCC-281), *A. flavus* (MTCC-1973), and *A. terreus* (MTCC-1782).

Screening studies have demonstrated that the newly synthesized compounds have promising antibacterial and antifungal properties. Therefore, it was concluded that there exists a better scope for further study on this class of compounds.

Antitubercular activity

The results of the antitubercular evaluation results are given in Table 2. Newly synthesized compounds (4a-c) were assayed for inhibitory activity toward *M. tuberculosis* H37Rv (ATCC2794). The MIC expressed as µg/mL was determined for each compound.

The compound 4a showed excellent activity against *M. tuberculosis* H37Rv (MIC = 3.125 µg/mL) than the standards pyrazinamide and streptomycin (MIC = 3.125 and 6.25 µg/mL). The structure-activity relationship studies revealed that the presence of electron-withdrawing group chlorine at C-5 indole system may be attributed for enhanced antitubercular activity in the series and has emerged as promising antitubercular agents.

CONCLUSION

In the present study, indolyl-thiopyrimidine derivatives were synthesized using a conventional method. A rapid, improved, and eco-friendly synthesis of 1,2,3,4-tetrahydro-4-oxo-6-(5-substituted 2-phenyl-1*H*-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile (4a-c). Screening studies have demonstrated that the newly synthesized compound 4a exhibited promising antimicrobial and antitubercular properties. Therefore, it was concluded that there exists a better scope for further study on this class of compounds.

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AUTHORS' CONTRIBUTIONS

The study was designed and executed, all the experimental section, results and discussion, and analysis section were performed, editing and reviewing were done by Dr. Prabhaker Walmik

CONFLICTS OF INTEREST

There are no conflicts to declare.

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