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Design of an efficient three component one-pot synthesis of -1,2,3,4-tetrahydro-4-oxo-6-(5-substituted 2-phenyl-1*H*-indol-3-yl)-2-thioxopyrimidine-5-carbonitrile as antimicrobial and antitubercular agent

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In the present study, a novel, rapid, improved and eco-friendly synthesis of indolyl-pyrazolo-pyrimidine derivatives **4a-c** by using conventional method *via* one-pot multi-component reaction has been described. The structures of all these unknown compounds have been confirmed with the help of physical and spectroscopic techniques like IR, ^{1}H and ^{13}C NMR and mass spectrometry and these newly synthesized compounds have been evaluated for *in vitro* antimicrobial and antitubercular and antioxidant activities. The results reveal that compound **4a** exhibits promising antimicrobial, antitubercular and antioxidant properties when compared to the standard drugs.

Keywords: Indole, Pyrazole, Pyrimidin-2-one, Antimicrobial activity, Antitubercular activity

Heterocyclic compounds have been proved to be the most useful ones due to their possible practical applications, their diverse structural patterns and 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 *viz.*, anti-inflammatory[9,10], anticonvulsant[11], cardiovascular[12], antibacterial[13], COX-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 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. Fused pyrimidines have also been attracted considerable interest in medicinal chemistry research due to their versatility and a broad bioactive potential. 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]. Also substituted pyrimidine at position-2 or -4 with an amino group are 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 posse's antibacterial[18], antifungal[19], antileishmanial[20], anti-inflammatory[21], 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 on the synthesis of biologically active molecules[26-33]. Encouraged by the diverse biological activities of indole and pyrazole and pyrimidine heterocyclic compounds and it was decided to prepare a new series of indolyl-pyrazolo-pyrimidine derivatives **3a-c**. Literature survey revealed that incorporation of different groups in one frame *i.e.*, indole, pyrazole and pyrimidin-2-one, heterocycles it may leads enhanced antimicrobial, antitubercular and antioxidant activities.

Results and Discussion

Chemistry

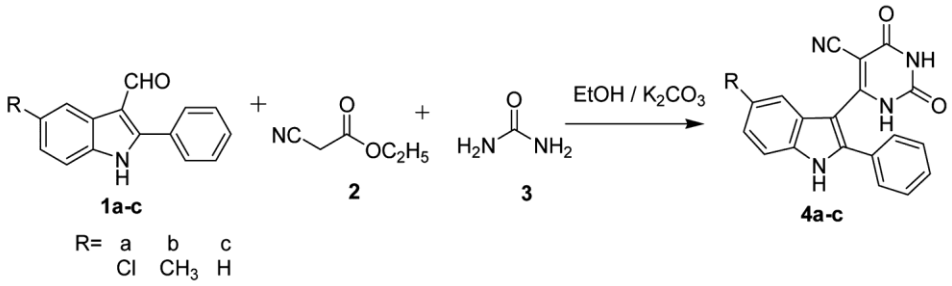
In the present study, indolyl-pyrazolo-pyrimidine derivatives **3a-c** were synthesized by using conventional method. A rapid, improved and eco-friendly synthesis of thiopyrimidines is carried out *via* one-pot mutlticomponent reaction of 5-chloro 2-phenyl indole-3-carboxaldehyde **1a**, ethylcyanoacetate **2** and thiourea **3** in the presence of ethanolic K_2CO_3 using conventional method to gave 1,2,3,4-tetrahydro-4-oxo-6-(5-chloro-2-phenyl-1*H*-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 carbony group of pyrimidne nucleus. The IR absorption band at 1108 cm^{-1} which corresponds to the C=S stretching supports the formation of compound **4a**. ^{1}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 at δ 10.08 and 10.01, due to the two–NH protons of thiopyrimidine, respectively. The aromatic protons resonated as multiplet around δ 7.30-7.71, these proton NMR data support the formation of compound **4a**. Mass spectral data shows that the isotopic peaks at m/z 378.034 (M^+), 380.036 (M^++2); which clearly confirm the formation of compound **4a**. Similarly, other compounds **4b** and **4c** in the series were confirmed (Scheme 1). The results were given in the material and method section.

Biological Activities

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 µg/mL which is comparatively more or equipotent than the standards gentamycicn and fluconazole.

Antibacterial activity of screened samples, compound **4a** showed potent activity (62.5 µg/mL) against *Escherichia coli* (MTCC-723), Antifungal activity screening results revealed that the compound **4a** showed potent activity (62.5 µg/mL) against *Aspergillus niger* (MTCC-281), (125 µg/mL) *Klebsiella pneumonia* (NCTC-13368), this potent activity may be due to presence of chlorine atom at C-5 position of indole system. Compound **4a** exhibited equipotent activity against *Staphylococcus aureus* (ATCC-29513), *Pseudomonas aeruginosa* (MTCC-1688). Antibacterial study revealed that the compound **4a** exhibited equipotent activity against all tested bacteria *Aspergillus oryzae* (MTCC-3567^T), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973), *Aspergillus terreus* (MTCC-1782).



Scheme 1 — Schematic pathway for synthesis of compounds **4a-c**

Table 1 — *In vitro* antimicrobial activities of compounds **4a-c**

Compound	Antibacterial activity (MIC, µg/mL)				Antifungal activity (MIC, µg/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

[a]EC- *Escherichia coli* (MTCC-723), [b]SA- *Staphylococcus aureus* (ATCC-29513), [c]KP- *Klebsiella pneumonia* (NCTC-13368), [d]PA- *Pseudomonas aeruginosa* (MTCC-1688) [e]AO- *Aspergillus oryzae* (MTCC-3567^T), [f]AN- *Aspergillus niger* (MTCC-281), [g]AF- *Aspergillus flavus* (MTCC-1973), [h]AT- *Aspergillus terreus* (MTCC-1782)

Table 2 — Antitubercular activity of compounds **4a-c**

Compd	MIC[a] values (μg/mL)
4a	3.125
4b	12.5
4c	25
Pyrazinamide	3.125
Streptomycin	6.25

Antitubercular activity

The results of the antitubercular evaluation results are given in Table 2. Newly synthesized compounds (**4a-c**) were assayed for inhibitory activity towards *Mycobacterium tuberculosis* H37Rv (ATCC2794). The minimum inhibitory concentration (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 (SAR) studies revealed that the presence of electron withdrawing group chlorine at C-5 indole system may be attributed as promising antitubercular agent.

Experimental Section

Chemistry

Materials and 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 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) FT-IR Spectrometer. The ^{1}H and ^{13}C NMR (DMSO-d_6) spectra were recorded with a Bruker NMR 500 and 125 MHz spectrometers, and the chemical shift values are expressed in δ (ppm) 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-1*H*-indol-3-carboxaldehydes, 1a-c

1a-c were prepared by following literature method[34].

General procedure for the 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

Appropriate mixture of 5-substituted 2-phenyl indole-3-carboxadehydes (**1a-c**) (0.01 mol), ethylcyanoacetate **2** (0.01 mol) and urea **3** (0.01 mol) in ethanol (25 mL) containing and potassium carbonate (0.01 mol) was taken in round bottom flask, and 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 recrystalized with ethanol was to afforded **4a-c**.

6-(5-Chloro-2-phenyl-1*H*-indol-3-yl)-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-5-carbonitrile, 4a: Yield 91%. m.p. 221°C. R_f 0.83 ethyl acetate: benzene (1:1) mixture; FT-IR (KBr): 3319 (CO-NH-CS), 3212 (CONH) 3109 (indole-NH), 2201 (CN), 1668 (CO), 1661 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6): δ 12. 01 (s, 1H, indole NH), 10.11 (s, 1H,CO-NH), 9.96 (s, 1H, NH-CS),7.30-7.73 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d_6): δ 169.9, 162.7, 151.5, 133.6, 129.5, 126.8, 123.8, 121.6, 119.4, 115.8, 112.1, 104 and 80.1; MS (EI): *m/z* 362.057 (M^+), 364.088 (M^++2). Anal. Calcd for $C_{19}H_{11}N_4O_2Cl$ (362.057): C, 62.91; H, 3.06; N, 15.44. Found: C, 62.98; H, 3.01; N, 15.42%.

6-(5-Methyl-2-phenyl-1*H*-indol-3-yl)-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-5-carbonitrile, 4b: Yield 84%. m.p. 207-08°C. R_f 0.86 ethyl acetate: benzene (1:1) mixture; FT-IR (KBr): 3321 (CO-NH-CS), 3210 (CONH) 3109 (indole-NH), 2209 (CN), 1665 (CO), 1662 cm^{-1} (CO); ^{1}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),7.26-7.771 (m, 8H, Ar-H), 2.47 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 169.5, 162.9, 151.6, 134.1, 130.2, 126.2, 123.9, 122.1, 120.6, 116.8, 112.6, 105 , 80.6 and 24.8; MS (EI): *m/z* 342.112 (M^+). Anal. Calcd for $C_{20}H_{14}N_4O_2$ (342.112): C, 70.17; H, 4.12; N, 16.37. Found: C, 70.22; H, 4.11; N, 16.39%.

6-(2-Phenyl-1*H*-indol-3-yl)-1,2,3,4-tetrahydro-2,4-dioxopyrimidine-5-carbonitrile, 4c: Yield 88%. m.p. 201-02°C. R_f 0.87 ethyl acetate:benzene (1:1) mixture; FT-IR (KBr): 3332 (CO-NH-CS), 3218 (CONH) 3111 (indole-NH), 2197 (CN), 1669 (CO), 1664 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6): δ 12. 09 (s, 1H, indole NH), 10.11 (s, 1H, CO-NH), 10.06 (s, 1H, CONH), 7.31-7.93 (m, 9H, Ar-H); ^{13}C NMR (DMSO-

d_6): δ 170.2, 162.9, 151.8, 134.6, 130.25 126.8, 123.9, 122.2, 121.7, 117.9, 112.5, 104.2 & 80.6; MS (EI): *m/z* 344 (M^+). Anal. Calcd for $C_{19}H_{12}N_4O_2$ (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 micro dilution method[35] in DMF at concentration 500, 250, 125 and 62.5 µg/mL. Muller Hinton broth was used as nutrient medium to growth and dilutes the compound suspension for the test bacteria and Saboured Dextrose broth used for fungal nutrition. Inoculums size for test strain was adjusted to 10^8 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strain employed for the activity was procured from Department of Biotechnolgy, Sahyadri Science College, Shivamogga.

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-3567^T), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973) and *Aspergillus terreus* (MTCC-1782). DMSO was used as a vehicle to get desired concentration of compounds to test upon microbial strains. The lowest concentration which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotic used for comparison in 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 *M. tuberculosis* $H37R_V$ strain using micro plate alamar blue dye assay (MABA)[36]. Briefly, 200 µL of sterile de-ionzed 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 to 0.2 µg/mL and compared with standards pyrazinamide 3.125 µg/mL and streptomycin 6.25 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, 25 µL freshly prepared 1:1 mixture of almar blue reagent and 10% tween-80 was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC (Minimal inhibition concentration) was defined as lowest drug concentration which prevented the colour change from blue to pink. The results are shown in Table 2.

Conclusion

In the present study, a novel, 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)** by using conventional method *via* one-pot mutlticomponent reaction. The biological screening studies have demonstrated that the newly synthesized compound **4a** exhibited promising antimicrobial and antitubercular properties. Therefore, it was concluded that there exists better scope for further study on this class of compounds.

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