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Research Article

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, ^{1}H, and ^{13}C 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], cyclo-oxygenase-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 synthesize of indole and pyrimidine nucleus embedded in one frame (indolyl-pyrimidine) to get a more potent molecule and testing for anti-microbial and anti-anticancer 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 ^{1}H and ^{13}C NMR (dimethyl sulfoxide [DMSO]-d_6) 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-1*H*-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-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 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}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}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 $C_{19}H_{11}N_4OSCl$ (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-1*H*-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}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}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 $C_{20}H_{14}N_4OS$ (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-1*H*-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}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}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 $C_{19}H_{12}N_4OS$ (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 μg/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. Inoculums 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-3567^T), *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 deionzed 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 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 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-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 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}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.

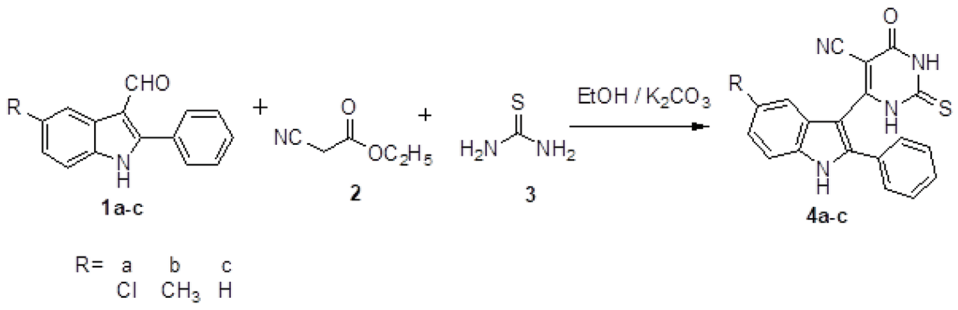
Antimicrobial activity

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 μg/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 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)



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^T), *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.

REFERENCES

1. Ansari KF, Lal C. Synthesis and biological activity of some heterocyclic compounds containing benzimidazole and beta-lactam moiety. J Chem Sci 2009;121:1017-25.
2. Antus S, Gulácsi K, Juhász L, Kiss L, Kurtán T. Synthesis of naturally occurring o-heterocyclic compounds of biological activity. Pure Appl Chem 2004;76:1025-32.
3. Mostafa TB. Synthesis and modification of some heterocyclic compounds with potential biological activity coupled on poly (maleic anhydride–methyl methacrylate). J Am Sci 2010;6:512-24.
4. Singh AK, Mishra G, Jyoti K. Review on biological activities of 1,3,4-thiadiazole derivatives. J Appl Pharm Sci 2011;1:44-9.
5. Salimon J, Salih N, Hussien H, Yousif E. Synthesis and characterization of new heterocyclic compounds derived from 2-aminopyridine. Eur J Sci Res 2009;31:256-64.
6. Xu PF, Zhang ZH, Hui XP, Zhang ZY, Zheng RL. Synthesis of triazoles, oxadiazoles and condensed heterocyclic compounds containing cinchopheny and studies on biological activity of representative compounds. J Chin Chem Soc 2004;51:315-9.
7. Bagley MC, Lubinu MC. Microwave-assisted multicomponent reactions for the synthesis of heterocycles. J Heterocycl Chem 2006;1:31-58.
8. Biswa MS, Ravi Kumar BV, Jnyanaranjan P, Dinda SC. Ecofriendly and facile one-pot multicomponent synthesis of thiopyrimidines under microwave irradiation. J Nanopart 2013;2013:780786.
9. Misra U, Hitkari A, Saxena AK, Gurtu S, Shanker K. Biologically active indolylmethyl-1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 4H--1,3,4-triazoles and 1,2,4-triazines. Eur J Med Chem 1996;31:629-35.
10. Preeti R, Srivastava VK, Ashok KV. Synthesis and antiinflammatory activity of heterocyclic indole derivatives. Eur J Med Chem 2004;39:449-52.
11. El-Gendy Adel A, Abdou Naida A, Sarhan El-Taher Z, El-Banna Hosney AY. Synthesis and biological activity of functionalized indole-2-carboxylates, triazino- and pyridazino-indoles. Alex J Pharm Sci 1993;7:99-5.
12. Kumar A, Saxena KK, Gurtu S, Sinha JN, Shanker K. Indole alkaloids have been proved to be medicinally important natural durg. Indian Drugs 1986;24:1-6.

13. Yamamoto Y, Kurazono M. A new class of anti-MRSA and anti-VRE agents: Preparation and antibacterial activities of indole-containing compounds. Bioorg Med Chem Lett 2007;17:1626-8.
14. Süreyya O, Doğu N. Synthesis and biological evaluation of N-substituted indole esters as inhibitors of cyclo-oxygenase-2 (COX-2). Farmaco 2002;57:677-83.
15. Chen JJ, Wei Y, Williums JD, Drach JC, Townsend LB. Design, synthesis, and antiviral evaluation of some polyhalogenated indole C-nucleosides. Nucleosides Nucleotides Nucleic Acids 2005;24:1417-37.
16. Baba M, Pauwels R, Herdewijin P, Clerq DE, Desmyter J, Vandeputte M. Both 2',3'-dideoxythymidine and its 2',3'-unsaturated derivative (2',3'-dideoxythymidinene) are potent and selective inhibitors of human immunodeficiency virus replication *in vitro*. Biochem Biophys Res Commun 1987;142:128-34.
17. Sharma P, Rane N, Gurram VK. Synthesis and QSAR studies of pyrimido[4,5-d]pyrimidine-2,5-dione derivatives as potential antimicrobial agents. Bioorg Med Chem Lett 2004;14:4185-90.
18. Ito S, Masuda K, Kusano S. Pyrimidine derivative, process for preparing same and agricultural or horticultural fungicidal composition containing same. U S Patent 4 1991;988:704.
19. Ram VJ, Haque N, Guru PY. Chemotherapeutic agents XXV: Synthesis and leishmanicidal activity of carbazolylpyrimidines. Eur J Med Chem 1992;27:851-5.
20. Amir M, Javed SA, Kumar H. Pyrimidine as antiinflammatory agent: A review. Indian J Pharm Sci 2007;68:337-41.
21. Vega S, Alonso J, Diaz JA, Junquera F. Synthesis of 3-substituted-4-phenyl-2-thioxo-1,2,3,4,5,6,7,8-octahydrobenzo[4,5]thieno[2,3-á] pyrimidines. J Heterocycl Chem 1990;27:269-73.
22. El-Kerdawy MM, Yousif Y, El-Emam AA, Moustafa MA, El-Sherbeny MA. Synthesis and antiinflammatory activity of certain thienopyrimidine derivatives. Boll Chim Farm 1996;4:301-5.
23. Modica M, Santagati M, Santagati A, Cutuli V, Mangano N, Caruso A. Synthesis of new [1,3,4]thiadiazolo[3,2-a]thieno[2,3-d]pyrimidinone derivatives with antiinflammatory activity. Pharmazie 2000;55:500-2.
24. Chambhare RV, Khadse BG, Bobde AS, Bahekar RH. Synthesis and preliminary evaluation of some N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d]pyrimidin-3-yl]-carboxamide and 3-substituted-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d]pyrimidin-4-ones as antimicrobial agents. Eur J Med Chem 2003;38:89-100.
25. Santagati NA, Caruso A, Cutuli VM, Caccamo F. Synthesis and pharmacological evaluation of thieno[2,3-d]pyrimidin-2,4-dione and 5H-pyrimido [5,4-b]indol-2,4-dione derivatives. Farmaco 1995;50:689-95.
26. Prabhaker W, Basavaraj SN, Swathi B, Somashekhar G. Design, synthesis of biologically active heterocycles containing indol- thiazolyl-thiazolidinone derivatives. Int J Pharm Pharm Sci 2018;6:113-7.
27. Prabhaker W, Saundane AR. Synthesis of novel indolyl-thiazolidinone derivatives as antioxidant, antimicrobial and atitubercular agents. Der Pharm Chem 2015;7:131-40.
28. Prabhaker W, Saundane AR. Synthesis of novel indolyl-azetidinone and thiazolidinone derivatives as a potent antioxidant, antimicrobial and antitubercular agents. Der Pharm Chem 2014;6:70-9.
29. Saundane AR, Yarlakatti M, Prabhaker W, Katkar V. Synthesis, antioxidant, antimicrobial, antimycobacterial, and cytotoxic activities of azetidinone and thiazolidinone moieties linked to indole nucleus. J Chem Sci 2012;124:469-81.
30. Saundane AR, Prabhaker W, Yarlakatti M, Katkar V, Vaijeenath AV. Synthesis and biological activities of some new annulated Pyrazolopyranopyrimidines and their derivatives containing indole nucleus. J Heterocycl Chem 2014;51:303-14.
31. Saundane AR, Prabhaker W, Kirankumar N M, Annapurna H. Synthesis of novel N-(aryl) diazenyl thiazol-2-amines and bezylidenethiazolidin-4-ones linked to indole nucleus as antioxidant, antimicrobial, antimycobacterial and cytotoxic agents. Int J Pharm Pharm Sci 2014;6:141-7.
32. Saundane AR, Prabhaker W. Synthesis, antioxidant, antimicrobial, antimycobacterial, and cytotoxic activities of azetidinone and thiazolidinone moieties linked to indole nucleus. J Chem 2013;2013:543815.
33. Saundane AR, Prabhaker W. Synthesis, antimicrobial and antioxidant activities of some indole analogues containing naphthyridine and pyrimidonaphthyridine systems. Indian J Chem 2012;51B:1593-6.
34. Hiremath SP, Biradar JS, Purohit MG. A new route to indolo [3,2-b] isoquinolines. Indian J Chem 1982;21B:249-53.
35. National Committee for Clinical Laboratory Standards. M100 Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement. Wayne, Pennsylvania: National Committee for Clinical Laboratory Standards; 2002.
36. Maria CS, De Souza MV, Alessandra CP, Marcelle LF, Raoni SB, Thais CM, *et al*. Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. ARKIVOC 2007;15:181-91.