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SYNTHESIS, ANTIMICROBIAL ACTIVITIES OF SOME NOVEL PYRIDINE AND PYRIMIDINE DERIVATIVES

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ABSTRACT

The main aim of the work was to develop antimicrobial active compounds. As a part of systematic investigation of synthesis and biological activities of heterocyclic compounds containing pyrazole, quinoline, salicyaldehyde moieties linked to various heterocyclic systems. We have synthesized new compounds *viz.*, 2-amino-4-(substituted)-6-phenylnicotinonitriles (**3a-c**), 4-(substituted)-6-phenyl-1H-pyrazolo[3,4-b]pyridin-3-amines (**4a-c**) and 5-(substituted)-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dithiones (**5a-c**). Structures of these compounds have been deduced upon the basis of elemental analysis and spectral data (IR, NMR and Mass). Antibacterial activity results revealed that, compounds **4a, 4b** and **5a** showed promising activity versus *P. Aeruginosa*, whereas antifungal activity results indicated that, compounds **4b, 5a** and **5c** exhibited maximum zone of inhibition against *A. Niger*.

KEYWORDS: Pyrazole, quinoline, pyridine, pyridopyrimidine, pyrazolopyridine, antimicrobial.

INTRODUCTION

Heterocyclic compounds are acquiring more importance in recent years because of their broad biological and pharmacological properties. Nitrogen, sulphur or oxygen containing five or six member is one of the most fruitful and occupied enormous significance in the field of medicinal chemistry. Molecules containing pyridine always have grave importance in chemistry as well as in biology. The pyridine substructure is one of the most prevalent

heterocyclic molecular frameworks found in natural products, pharmaceuticals, vitamins and functional materials.[1-4] The privileged nature of pyrimidine structure in terms of their shape and hydrogen bonding characteristics make them ideal starting points in the search of new chemical entities of biological activities such as antimicrobial,[5] antitubercular[6] and antioxidant activities, etc.[7]

Fused heterocyclic systems containing pyrazole ring are ranked among the most versatile bioactive compounds. Pyrazolopyridine is an example of such fused system, which is known to possess remarkable and significant biological and medicinal importance, viz., antibacterial,[8] antiviral.[9] and antioxidant[10] activities. In addition, pyridopyrimidine derivatives display potentially useful biological activities, antibacterial,[11] and antitumor activities[12] etc.

In our previous communication,[13] we have reported the synthesis and antimicrobial activities of different heterocyclic compounds containing pyrazole, quinoline and salicyaldehyde moieties, in which 2-amino-3-cyano-4H-pyran (**1**), pyrano[2,3-d]pyrimidin-2(5H)-one/thione (**2**) and pyrano[2,3-c]pyrazol-3-amine (**3**) (Fig-1). Prompted by these results and in continuation of our research work in the synthesis of biologically important heterocyclic compounds, we herein report the synthesis of title compounds in which pyran system is replaced by the pyridine nucleus in the below heterocycles (**1-3**) to evaluate the impact of these compounds on their antimicrobial activities.

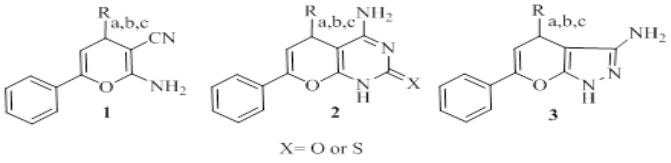
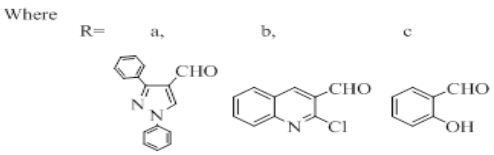


Fig-1: Motivation for synthesis of antibacterial active compounds



MATERIALS AND METHODS

All the reagents were obtained commercially and used by further purification. 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 NMR (DMSO-d_6) spectra were recorded with a BRUKER NMR 500 MHz spectrometer, 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. All the compounds gave C, H and N analysis within ± 0.4%.

General procedure for synthesis of 1, 3-diphenyl-1H-pyrazole-4-carboxyaldehyde (1a)

5.4 ml of phenyl hydrazine (0.01 mol) and 6 ml of acetophenones (0.01 mol) are taken in a round bottom flask and heated for 15 min, add 20 ml of DMF and shake then prepare Vilsmeier reagent [i.e by mixing phosphorous oxychloride and dimethyl formamide (0.03 mol) each] maintaining a temperature of 0-5^{0}C, add the reagents to the round bottom flask drop wise by cooling and reflux for 5 hrs. Basify the solution with 25% ammonia, solid separated is filtered and washed with water, dried and recrystallized to furnish **(1a).**

General procedure for synthesis of 2-chloroquinoline-3-carboxyaldehyde (1b)

Dimethyl formamide (0.06 mol) was cooled to 0^{0}C in flask equipped with a drying tube and phosphorous oxychloride (0.06 mol) was added drop wise with constant stirring at room temperature. To this solution acetanilide (0.01 mol) was added in small portions and after 5 min, the reaction mixture was refluxed for 16 hrs on boiling water bath. The reaction mixture was decomposed in crushed ice and stirred for 30 min. The solid separated was filtered, washed with water, dried and recrystallized to furnish **(1b).**

General procedure for synthesis of 3-(substituted)-1-phenylprop-2-en-1-ones (2a-c)

A solution of (**1a-c**) (0.01 mol) and acetophenone (0.01 mol) in 20 ml of ethanol was treated with 20 ml of 60% KOH solution at 5-10^{0}C. The reaction mixture was stirred at RT for 4 hrs. It was then diluted with water (20 ml) and extracted with diehyl ether (3 x 20 ml). The aqueous solution was acidified with dilute HCl. The solid obtained was filtered, washed with water and dried. The crude product was purified by recrystallization to furnish **(2a-c).**

General procedure for synthesis of 2-amino-4-(substituted)-6-phenylnicotinonitriles (3a-c)

To solution of **2a-c** (0.01 mol) in acetic acid malononitrile (0.01 mol) and ammonium acetate (0.01 mol) were added and reaction mixture was refluxed for 5 hrs. The reaction mixture was

cooled to room temperature and decomposed in ice cold water. The solid separated was filtered off, dried and purified in ethanol to furnish **(3a-c).**

General procedure for the synthesis of 4-substituted-6-phenyl-1H-pyrazolo [3,4-b]pyridin-3-amines (4a-c)

A solution of **3a-c** (0.01 mol) and hydrazine hydrate (99%, 0.03 mol) in ethanol (5 ml) was heated under reflux for 3 hrs. After cooling the product separated was filter, washed with cold ethanol and recrystallized from ethyl acetate to yield compounds (**4a-c)**.

General procedure for the synthesis of 5-substituted-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dithiones (5a-c)

A mixture of compounds **3a-c** (0.01 mol) and carbon disulfide (0.04 mol) in pyridine (15 ml) was refluxed on water bath for 10-15 hrs, and excess of pyridine was removed under vacuum. The reaction mixture was cooled to room temperature and poured in to cold water. The product thus separated was filtered, washed with water, saturated with $NaHCO_3$ solution followed by water and recrystallized from benzene to get pure **5a-c**.

RESULT AND DISCUSSION

The precursor 3-(substituted)-1-phenylprop-2-en-1-ones (**2a-c**) was synthesized by treatment of various aldehydes (**1a-c**) with acetophenone (0.01 mol) and KOH (**scheme-1**).

Compound (**2a)** when subjected to heterocyclization with malononitrile and ammonium acetate in presence of a catalyst triethylamine in refluxing acetic acid yielded 2-amino-4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-phenylnicotinonitrile (**3a)**.[13]

Compound **3a** on cyclocondensation with hydrazine hydrate in alcohol at reflux temperature afforded 4-(1,3-diphenyl-1H-pyrazol-4-yl)-6-phenyl-1H-pyrazolo [3,4-b]pyridin-3-amine (**4a**). The IR spectrum of compound **4a** showed absorption bands at 3318, 3255 and 1592 cm^{-1} due to (NH_2), pyrazole-NH and (C=N) functions, respectively, whereas absorption due to (CN) function at 2274 cm^{-1} was missing. In its ^1H NMR spectrum, the signals revealed at δ 9.00 (s, 1H, pyrazole-NH), 6.95-7.81 (m, 16H, Ar-H), 6.00 (s, 2H, NH_2) and 5.10 (s, 1H, pyrazole-CH). The mass spectrum of the compound exhibited molecular ion peak at m/z 428.

Compound **3a** when subjected to cyclocondensation with carbon disulfide in pyridine under reflux condition afforded 5-(1,3-diphenyl-1H-pyrazol-4-yl)-7-phenylpyrido [2,3-d]pyrimidine-2,4(1H,3H)-dithione (**5a)**. In its IR spectrum exhibited characteristic absorption

bands at 3305, 3185, 1601, 1198 and 1020 due to NH, NH, C=N, C=S and C=S respectively, whereas the absorption due to the (CN) function at 2274 cm^{-1} was missing. In its ^1H NMR spectrum, the signals revealed at δ 10.98 (s, 1H, pyrimidin-NH), 10.80 (s, 1H, pyrimidin-NH), 7.00-8.00 (m, 16H, Ar-H) and 5.38 (s, 1H, pyrazole-CH). The mass spectrum of **5a** exhibited isotopic molecular ion peak at m/z 489. Physical and spectral data of other compounds are given in Table-1 and 2.

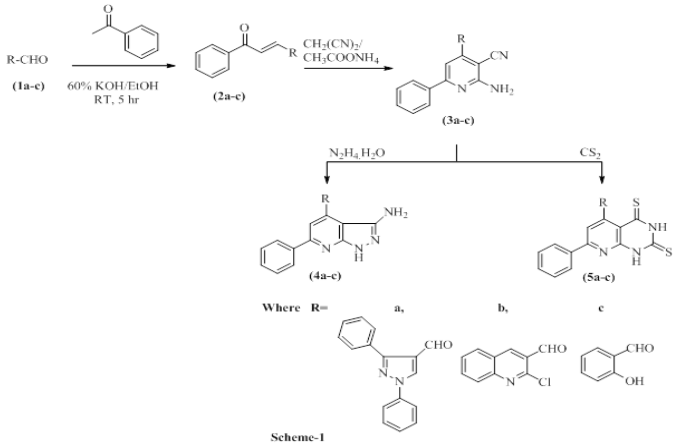


Table-1: Physical data of synthesized compounds (4-5)

Comp No	Substitution R	Molecular formula	Yield (%)	M. P. (°C)	Elemental Analysis. Calculated (found)		
					C	H	N
4a		$C_{27}H_{20}N_6$	72	275-76	75.68 (75.65)	4.70 (4.67)	19.61 (19.65)
4b		$C_{21}H_{14}ClN_5$	63	225-26	67.83 (67.87)	3.80 (3.76)	18.84 (18.79)
4c		$C_{18}H_{14}N_4O$	75	210-11	71.51 (71.49)	4.67 (4.63)	18.53 (18.55)
5a		$C_{28}H_{19}N_5S_2$	80	282-83	68.69 (68.64)	3.91 (3.93)	14.30 (14.33)
5b		$C_{22}H_{13}ClN_4S_2$	59	250-51	61.03 (61.00)	3.03 (3.06)	12.94 (12.90)
5c		$C_{19}H_{13}N_3OS_2$	66	294-95	62.79 (62.77)	3.61 (3.64)	11.56 (11.59)

Table No-2: Spectral data of synthesized compounds (4-5)

Comp No	IR(KBr) cm^{-1}	^1H NMR (DMSO) δ ppm	MS (M/z) m/z[M^+]
4a	3318 (NH_2), 3255 (NH), 1592 (C=N)	9.00 (s, 1H, pyrazole NH), 6.95-7.81 (m, 16H, Ar-H), 6.00 (s, 2H, NH_2), 5.10 (s, 1H, pyrazole-CH)	428

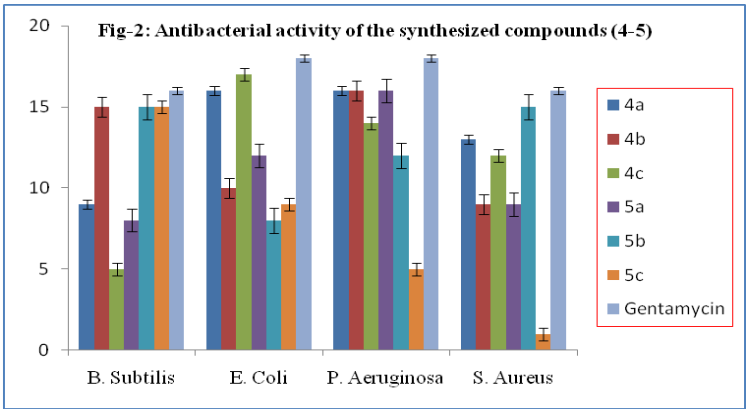
4b	3346 (NH_2), 3260 (NH), 1600 (C=N)	8.80 (s, 1H, pyrazole NH), 7.12-8.05 (m, 11H, Ar-H), 5.50 (s, 2H, NH_2)	371, 373
4c	3345 (OH), 3128 (NH), 3092 (NH_2), 1595 (C=N)	9. 60 (s, 1H, pyrazole NH), 9.10 (s, 1H, OH), 7.00-8.01 (m, 10H, Ar-H), 5.48 (s, 2H, NH_2)	302
5a	3305 (NH), 3185 (NH), 1601 (C=N), 1198 (C=S), 1020 (C=S)	10.98 (s, 1H, pyrimidine-NH), 10.80 (s, 1H, pyrimidine-NH), 7.00-8.00 (m, 16H, Ar-H), 5.38 (s, 1H, pyrazole-CH)	489
5b	3328 (NH), 3298 (NH), 1595 (C=N), 1163 (C=S), 1014 (C=S)	10.50 (s, 1H, pyrimidine-NH), 10.47 (s, 1H, pyrimidine-NH), 7.22-8.10 (m, 10H, Ar-H),	432, 434
5c	3310 (OH), 3200 (NH), 3160 (NH), 1603 (C=N), 1180 (C=S), 1008 (C=S)	10.85 (s, 1H, pyrimidine-NH), 10.71 (s, 1H, pyrimidine-NH), 10.00 (s, 1H, OH), 7.00-8.00 (m, 10H, Ar-H),	363

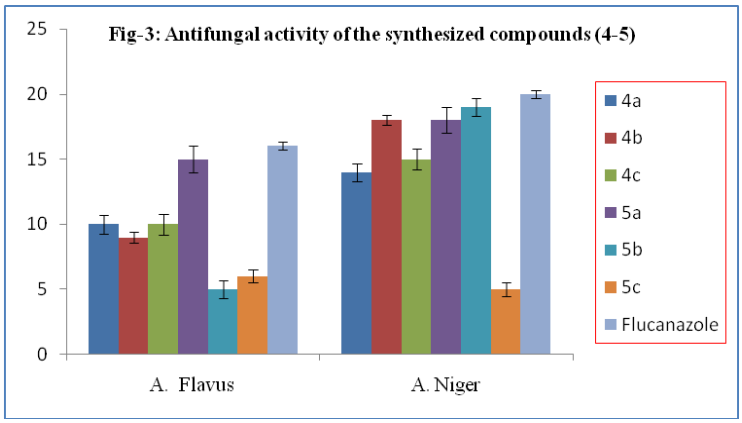
ANTIMICROBIAL ACTIVITY

The antimicrobial activities were performed by cup plate method.[14] Antibacterial activity screened against *B. Subtilus*, *E. Coli.* *P. Aeruginos* and *S. Aureus.* Antifungal activity was carried out against *A. Flavus* and *A. Niger* under aseptic conditions using Gentamycine and Fluconazole as standard drug, respectively. The zone of inhibition was compared with standard drug after 24 hrs of incubation at 25^0C for antibacterial activity and 48 hrs at 30^0C for antifungal activity.

Antibacterial result (Fig-2) suggested that compounds **4b, 5b** and **5c** showed good activity against gram + ve bacteria *B. Substilus*, whereas compounds **4a** and **4c** exhibited maximum zone of inhibition against *E. Coli.* Compounds **4a**, **4b** and **5a** showed good activity against bacterial strain *P. Aeruginos.* Compound **5b** promising activity against *S. Aureus.*

Antifungal activity results (Fig-3) revealed that compound **5a** showed good activity against *A. Flavus.* Compounds **4b**, **5a** and **5b** exhibited good zone of inhibition against *A. Niger.* Remaining other compounds showed poor activity against all the bacterial and fungal strains compared with standards.





CONCLUSION

The present work reports the synthesis of novel 4-substituted-6-phenyl-1H-pyrazolo[3,4-b]pyridin-3-amines and 5-substituted-7-phenylpyrido[2,3-d]pyrimidine-2,4(1H,3H)-dithiones in simple reaction conditions and evaluated for antimicrobial activity. The antimicrobial activity suggests that some pyrazolopyridin and pyridopyrimidine analogs may lead to the discovery of novel class of molecules of hitherto unknown biological activity.

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REFERENCES

1. Jones G. Comprehensive heterocyclic chemistry II. pyridines and their benzo derivatives synthesis. ELSEVIER, 1996; 5. 05: 167-243.

2. Henry GD, De novo synthesis of substituted pyridines. Tetrahedron, 2004; 60(29): 6043-6061.
3. Joseph PM, Quinoline, quinazoline and acridone alkaloids. Natural Product Reports, 2005; 22(5): 627-646.
4. Movassaghi M, Hill MD, Ahmad OK. Direct synthesis of pyridine derivatives. Journal of the American Chemical Society, 2007; 129: 10096-10097.
5. Desai NC, Makwana AH, Senta RD. Synthesis, characterization and antimicrobial activity of some novel 4-(4-(arylamino)-6-(piperidin-1-yl)-1,3, 5-triazine-2-ylamino)-*N*-(pyrimidin-2-yl)benzenesulfonamides. Journal Saudi Chemical Society, 2015; 18(6): Doi:10.1016/J.Jscs.2015.01.004. [Article in Press].
6. Chikhale R, Menghani S, Babu R, Bansode R, Bhargavi G, Karodia N, et al. Development of selective DprE1 inhibitors: design, synthesis, crystal structure and antitubercular activity of benzothiazolylpyrimidine-5-carboxamides. European Journal Medicinal Chemistry, 2015; 96: 30-46.
7. Kotaiah Y, Nagaraju K, Harikrishna N, Rao CV, Yamini L, Vijjulatha M. Synthesis, docking and evaluation of antioxidant and antimicrobial activities of novel 1,2,4-triazolo[3,4-*b*] [1,3,4]thiadiazol-6-yl)selenopheno[2,3-*d*]pyrimidines. European Journal Medicinal Chemistry, 2014; 75: 95-202.
8. Ali A, Taylor GE, Ellsworth K, Harris G, Painter R, Silver LL, Young K. Novel pyrazolo[3,4-*d*]pyrimidine based inhibitors of *Staphlococcus Aureus* DNA polymerase III: design, synthesis, and biological evaluation. Journal Medicinal Chemistry**,** 2003; 46: 1824-1830.
9. Bernardino AM, Ferreira VF, Fontoura GAT, Frugulhetti ICPP, Lee MY, Romeiro GA, Souza MCBV, Sa P. Synthesis of 4-anilino-1*H*-pyrazolo[3,4-b]pyridine derivatives and their in vitro antiviral activities. Journal Brazil Chemical Society, 1996; 7: 273-277.
10. Saundane AR, Katkar Vijaykumar, Vaijinath AV, Prabhaker Walmik. Synthesis, antimicrobial and antioxidant activities of some new indole derivatives containing pyridopyrimidine and pyrazolopyridine moieties. Medicinal Chemistry Research, 2012; 19(7): 617-716.
11. Narayana LB, Ram Rao AR, Shanthan Rao P. Synthesis of new 2-substituted pyrido[2,3-d]pyrimidin-4(1H)-ones and their antibacterial activity. European Journal Medicinal Chemistry, 2009; 44(3): 1369-1376.

12. Zink M, Lanig H, Troschutz R. Structural variations of piritrexim, a lipophilic inhibitor of human dihydrofolate reductase: synthesis, antitumour activity and molecular modelling investigations. European Journal Medicinal Chemistry, 2004; 39: 1079-1088.
13. Goudgaon NM, Sheshikant BU, Vinod Tukaram, Synthesis and antimicrobial activity of novel 4-amino/oxo-5-substituted-7-phenylpyrido[2,3-*d*]pyrimidines, Journal of Pharmacy Research, 2014; 8(2): 205-211.
14. Kambe S, Saito K, Kishi H, Sakurai A, Midorikawa H, A one-step synthesis of 4-oxo-2-thioxopyrimidine derivatives by the ternary condensation of ethyl cyanoacetate, aldehyde and thiourea. Indian pharmacopoeia, Government of India (1985) 3rd Ed. New Delhi Appendix IV, 90. (1979) Synthesis 287-289.